# PRECLINICAL STUDY OF SIDDHA DRUG KOMOOTHIRA SILASATHU PARPAM'S HYPOGLYCEMIC, ANTI DYSLIPIDEMIC AND ANTI-OXIDANT ACTIVITIES

Dissertation submitted to

**THE TAMILNADU DR. MGR MEDICAL UNIVERSITY**

**CHENNAI-600032**

***In partial fulfilment of the requirements***

***for the award of the degree of***

**DOCTOR OF MEDICINE (SIDDHA)**

**BRANCH-II-GUNAPADAM**


**POST GRADUATE DEPARTMENT OF GUNAPADAM**

**THE GOVERNMENT SIDDHA MEDICAL COLLEGE**

**TIRUNELVELI-627002**

**OCTOBER 2019**

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# PALAYAMKOTTAI

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I hereby declare that this dissertation entitled **"Pre-clinical study of siddha formulation *'Komoothira silasathu parpam'* for its Hypoglycemic, Anti Dyslipidemic and anti-oxidant activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.G.Essakky pandian M.D(S),** Lecturer, Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai, Tirunelveli-02 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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# ACKNOWLEDGEMENT

I am extremely grateful to the Lord almighty that who empowered me with his blessings and grace to complete my dissertation work successfull..

I gratefully record my indebtedness to the respected **Vice Chancellor**, The Tamilnadu Dr.M.G.R.Medical University, Chennai and **Commissioner** of Indian Medicine and Homeopathy, Chennai.

I express my sincere thanks to our former Principal Prof.**Dr.R.Neelavathi, M.D(s), Ph.D.,** and Principal **Prof. Dr. S. Victoria M.D(S)** Govt. Siddha Medical College, Palayamkottai for his kind permission to carry out my research work.

It's my unique pleasure to express my hearted thanks to **Dr.A.Kingsly,M.D(S).,**Reader, Head of the department, P.G. Gunapadam Department, Govt siddha medical college, Palayamkottai for his excelled care, continuous support and optimisitic approach, which influenced me to accomplish this work successfully. I could never forget the help and priceless guidance throughout my life.

It gives me pride and pleasure to express my deep sense of gratitude to, my guide **Dr.G.Essakky Pandian,M.D(S).,** Lecturer, Government Siddha Medical college, Palayamkottai, for his constant support inspiring, invaluable guidance, and motivation that made me think and do this research work with confidence

It is with immense pleasure that I place on record my deep sense of gratitude to **Dr.R.Antony Duraichi, M.D(S).,** Lecturer Grade II, Department of P.G Gunapadam, Government Siddha Medical College, Palayamkottai, for his untiring consultation, encouragement during my research work. I am indebted to him for all his valuable and generosity.

I have not been still finding a suitable word to express my ineffable sense of gratitude to **Dr.M.Kalaivanan M.Sc, Ph.D.,** Department of Pharmacology, Govt. Siddha Medical College, Palayamkottai, for his constant help and encouragement to complete the pharmacological work successfully.

I am grateful to **Mrs.N.Nagaprema, M.Sc., M.Phil.,** and Head of the Department of Bio-chemistry, Govt.Siddha Medical College, and Palayamkottai for her kind help and suggestions on biochemical aspects of this dissertation.

I am very much happy to thank **Mrs. S.Sudha, M.Sc.,** Head of the Department of Herbal Botany and Herbal Pharmacognosy, Govt. Siddha Medical College, Palayamkottai for her kind help in botanical aspect of this study and valuable suggestions regarding drug identification.

I express my thanks to **Dr.R.Murugesan,M.Sc(Pharm), Ph.D.,** H.O.D, Department of pharmacology, Indian Institute of Technology (IIT),Chennai-36 for his valuable support in doing the Heavy metal analysis, scanning electron microscopic analysis of the trial drug.

I express my thanks to **Mr.M.Santhanakumar,** lecturer , Department of pharmacology, KLU College of pharmacy, coimbutore for the excellent help in pharmacology study.

I also acknowledge my thanks to **Dr.Thangamariyappan.,Ph.D**. **viveks laboratories Pvt ltd,** Nagercoil, for microbial analysis.

I extended my gratitude to the animal Ethical Committee Members for their approval to do animal studies in pre clinical studies.

I am also my thankful to our Librarian **Mrs.T.Poongodi,** M.Lib.Sc, M.Phil, and staffs for their kind co-operation for my study.

I am also thankful to **Mrs.Suganthi**, DMLT, Lab Technician , and Post Graduate Department of Gunapadam for her kind co-operation to purification and preparation of the trail drug for my study and successful completion of dissertation.

I should express my gratefulness thanks to my husband **M. Meenatchi Sundaram**, **B. Tech., MBA.**

I should express my gratefulness thanks to my lovable sister **M.Sanofer B. Sc.** for their help.

With immense pleasure I thank for the full support and co-operation given by my **Parents** and **all my friends** for the successful completion of this work.

Without the above, I might not be able to complete this dissertation as a successful one.

I owe everything to them. Besides this, several people have knowingly and unknowingly helped me in the successful completion of this project.

# ABBREVIATIONS

| | | |
|---|---|---|
| KSP | - | *Komoothira silasathu parpam* |
| DM | - | Diabetes Mellitus |
| CPCSEA | - | Committee For The Purpose Of Control And Supervision of Experimental Animal |
| ESR | - | Erythrocyte Sedimentation Rate |
| FTIR | - | Fourier Transform Infrared Spectroscopy |
| IAEC | - | Institutional Animal Ethical Committee |
| ICP-OES | - | Inductively Coupled Plasma Optical Emission Spectrometry |
| OECD | - | Organisation For Economic Co-Operation And Development |
| SEM | - | Scanning Electron Microscope |
| XRD | - | X-Ray Powder Diffraction |

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Name of the principle investigator : Dr. P.Revathy

Title of the Project : *Komoothira silajit parpam* for its hypoglycemic, hypolipidemic, anti oxidant acitivities

Proposal Number : AKCP/IAEC/14 ,40 / 2018-19

Date of received after modification (if any) : Nil

Date of received after second Modification : Nil

Approval date : 19.01.2018 &03.02.2018

Animals : Rats & Guinea pig

Expiry Date : Nil

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**Signature of IAEC Chairperson**

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| TAMIL NAME | ENGLISH NAME | CHEMICAL NAME |
|---|---|---|
| *KOMOOTHIRA SILAJIT* | ASPHALTUM | *ASPHALT MINERAL PITCH* |

Date: 4.7.2018
Station:

4/7/18
Authorised Signature.

Dr. G. ESSAKKYPANDIAN, MD(s)
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| TAMIL NAME | BOTANICAL NAME | FAMILY | PART USED |
|---|---|---|---|
| *KADUKKAI* | *Terminalia cheubula. Retz* | Combretaceae | Dried fruit |
| *THANDRI THOL* | *Terminalia bellarica. Roxb* | Combretaceae | Dried fruit |
| *NELLI MULLI* | *Emblica officinalis. Linn* | Euphorbiaceae | Dried fruit |

Date: 4.7.2018
Station: Palayamkottai

Authorised Signature

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# INTERNATIONAL JOURNAL OF REVERSE PHARMACOLOGY AND HEALTH RESEARCH

ISSN 2589 - 3343 *A Peer Reviewed Interdisciplinary Medical Journal*

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The board of "International Journal of Reverse Pharmacology and Health Research"
(ISSN 2589-3343, www.ijrphr.com) is herby awarding this certificate to Corresponding author

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***Review on Anti-diabetic action Medicinal Plants in Siddha Medicine***

CODENJ: IJRPHR

Published in Volume **2** , Issue **2** , Apr-Jun, **2019**

Council of Science Editors

STOCKHOLM UNIVERSITY PRESS

COPE COMMITTEE ON PUBLICATION ETHICS

Int. J. Rev. Pharmcol. Health Res. Editor-in-Chief www.ijrphr.com

Editor-in-Chief
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ISSN 9582-3343

# CERTIFICATE OF PUBLICATION

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## ANTIHYPERGLYCEMIC EFFECT OF KOMOOTHIRA SILASATHU PARPAM IN STREPTOZOTOCIN INDUCED DIABETIC RATS

in Volume 1 Issue 3 , 2019 (Jul-Sep) www.biosci.in/jrbms

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Dr. Jey Rituz

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# 1. INTRODUCTION

*Siddha* system is one of the traditional systems of medicine in India. In *Siddha* medicine, the individual is a microcosm of the universe. The human body consists of the five primordial elements - earth, water, fire, air and space, the three humours-*vatha, pitha* and *kapha* and seven physical constituents. The equilibrium of humours is considered as health and its disturbance or imbalance leads to a diseased state. Poet *Thiruvalluvar* has indicated the same view in this *Thirukural*,

"மிகினும் குறையினும் நோய்செய்யும் நூலோர்
வளி முதலா எண்ணிய மூன்று"

\- குறள் - 941

There is equal emphasis on the body, mind and spirit and strives to restore the innate harmony of the individual.

Treatment is aimed at restoring balance to the mind-body system. Diet, lifestyle, yoga and meditation play a major role not only in maintaining health but also in curing diseases.

**MATERIA MEDICA:**

This system has developed a rich and unique treasure of drug knowledge in which use of metals and minerals is very much advocated some idea about the depth of knowledge. The system possesses in the field of mineral, materia medica can be found from the detailed drug classification.

The system has classified separately classes of metals and alloys, which melt when heated and solidifies on cooling. These include items like gold, silver, copper, tin, lead and iron. These are incinerated by special processes and used in medicine.

There is a group of drugs that exhibit sublimation on heating and includes mercury and its different forms like red sulphite of mercury, mercuric chloride and red oxide of mercury etc.

Sulphur, which is insoluble in water, finds a crucial place in Siddha material medica along with mercury for use in therapeutics and in maintenance of health.

The above classification shows detailed knowledge and study of minerals that this system has evolved for treatment.

Siddha system that insists that the physician should enquire the nature of the disease, its cause and its method of cure and treat it faithfully *envagai thervugal* (the

eight methods of clinical examination) are used to determine the diagnosis, aetiology, treatment and prognosis of diseases.

In *Siddha* system of medicine, diseases are classified into 4448 types according to the *Siddhar 'yugimuni'*. Among them *Mehaneer noigal* is further classified into 21 types which include *Madhumegam*. *Madhumegam* is one of the challenging diseases in our modern life.

As per the literature *Siddha Maruthuvam* (*podhu*) author by *K.N. Kuppusamy mudhaliar* the disease *madhumegam* is defined as it is condition characterized by frequent profuse urination (*adikkadi siruneer peruvariyaga izhidhal*), often flies and ants are stacked to the urine (*neerizhindha idathil Ee, erumbugal arithal*) and on heating, it smells like sugar (*adhanai kaachin sarkarai manam veesal*) which is followed by emaciation of the body day by day (*udal naaluku naal izhaithal*).

In modern science *madhumegam* symptoms are may be correlated with diabetes mellitus.

Globally, an estimated 422 million adults are having diabetes mellitus, according to the latest 2016 data from the World Health Organisation (WHO). Diabetes prevalence is increases rapidly; previous 2013 estimates from the international Diabetes Federation put the number at 381 million people having diabetes. The number is projected to almost double by 2030. Type 2 diabetes makes up about 85-90% of all cases. Increases in the overall diabetes prevalence rates largely reflect an increase in risk factors for type 2, notably greater longevity and being overweight or as obese.

Diabetes mellitus throughout the world, but is more common(especially type 2) in the more developing countries. The greatest increase in prevalence is, however, occurring in low-and middle-income countries including in Asia and Africa, where most patients will probably be found by 2030. Prevalence of Diabetes Mellitus especially in India (98million) and 10.4% in Tamilnadu. The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, including increasingly sedentary lifestyles, less physically demanding work and the global nutrition transition, marked by increased intake of foods that are high energy-dense but nutrients-poor (often high in sugar and saturated fats, sometimes referred to as the western pattern diet). The risk of getting type 2 diabetes has been widely found to be associated with lower socio-economic position across countries.

WHO estimates that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death. However another 2.2 million deaths worldwide were attributable to high blood glucose and the increased risks of associated complications (e.g. heart disease, stroke, kidney failure), which often result in premature death and are often listed as the underlying cause of death certificates rather than diabetes.

Hence an effective and safer therapy is the need of current scenario, therefore an effort has been made to preclinically evaluate the '*KOMOOTHIRA SILASATHU PARPAM*' in the literature of*Thanjai Vaithiya Raja Sinthamani* part-2 page no. 5 which is exclusively indicated for *madhumegam*.

The mineral formulation especially parpam is superior than herbal formulation such as chooranam.

**பற்ப மகிமை**

வீரத்து மிக்கவை பற்பங்களே – பரி
காரத்து மிக்கவை பற்பங்களே
பாருக்குள் மானிடர் நோய்போக – வரு
பண்டிதருக்கெல்லா மாமோகம்
வீரகடாரி – பிணிக்கொரு
பாரகுடாரி – விசைபெறு
தீரதடாரி – வினையுடு
சூரிக்குழு நேரொத்தது
மேருக்கினை பாரப்புறம்

தேரையர் காப்பியம்

This dissertation is an endeavour to bring into spotlight the science behind the usage of *komoothira silasaathu parpam* in Siddha system to further reinforcement of traditional use for the treatment of *madhumegam* (diabetes mellitus*).*

# 2. AIM AND OBJECTIVES

**Aim:**

The aim of this study is to validate the safety and efficacy of '*komoothira silasathu parpam'* for "*madhumegam" (*diabetes mellitus) by pre-clinical studies.

**Objectives:**

The following methodology was adopted to evaluate the safety and efficacy of the test drug.

1. To collect the relevant classical *Siddha* literature as well as Modern Sciences that supports the study.
2. To standardize the preparation of the drug according to classical *siddha* literature.
3. To subject the drug into physico chemical & phyto chemical analysis.
4. To analyze the drug biochemically for the detection of acidic and basic radicals.
5. To Estimate the percentage of elements present in the drug by Instrumental analysis.
6. To Establish the following Pharmacological activities:
    - Hypoglycemia,
    - Anti Dyslipidemic,
    - Antioxidant
7. To evaluate the acute and sub-acute toxicity studies profile of *komoothira silasathu parpam* according to OECD guidelines.

# 3. REVIEW OF LITERATURE

## *3.1.* ASPHALTUM (*KOMOOTHIRA SILASATHU*)

### 3.1.1. Gunapadam aspect:

**Synonyms**:

"தாதுஜம் திதியினோடு தருசிலா சதுவுமாகும்
தீதறு மச் மஜந்தான் நிகழத்தினும் சைலேயம்மே
ஒது ஜத் வச் மஜம்மேயுறு கிரிஜாச்மஜப் பேர்
ஏதமில் நூலோர் கூறும் இருஞ் சிலாசத்துவின் பேராம்"

-- நிகண்டு ரத்னாகரம் 73, 208 பாடல்

*Thathujam, thithi, silasathu, achmajam, athirajam, salieyam, jath vaja majam, kinjach majam, peraicaicum, urainjiyam, silarasam, sayeleyam, kalkasilasathu.*

**VERNACULAR NAMES**:

| | | |
|---|---|---|
| English | : | Asphalt mineral pitch, Juv's pitch. |
| Sanskrit | : | *Silajit, Silaras.* |
| Gujarathi | : | *Silajata* |
| Malayalam | : | *Silajatu* |
| Kannadam | : | *Silajata* |
| Bengali | : | *Silajatu* |
| Arabic | : | *Hajar-ul-musa* |
| Persian | : | *Momiai, Faqurul Yahud* |
| Hindi | : | *Ral-Yahudi* |

**Organoleptic character:**

| | | |
|---|---|---|
| Colour | : | Black colour |
| Appearance | : | Black crystal |
| Taste | : | Bitter |

**Action:**

| External: | Internal: |
|---|---|
| Anthelmenitic | Tonic |
| Deobstruent | Laxative |
| Analgesic | Lithotriptic |
| Antiseptic | Diuretic |
| Deodorant | Expectorant |

**Origin:**

*Silasathu* is found in many mountain ranges of the world, at attitudes between 1000-5000 meters, namely in the Himalayas and Hindukush ranges of Indian subcontinent, Tibet mountains and in Haridhvar, Simla, Nepal, Hironia.

The word *silasathu* is composed of two parts *'sila'* means rock and *'sathu'* means having won. So it literally means conqueror of the mountains. It is blackishbrown material of variable consistency that exudes from layers of rock. *Komoothira silasathu* is one of the ingredients of *Attanga dhoopa pillai*.

"குங்கிலிய மகருவகியீக முடியந்தாறு
குறும்பொறை யிரத மணப்பால்"

**Types:**

சிலாசத்து வகைகள் :

பண்டென்ற சிலாசத்து வண்ணங் கேளு பாரமாகக் கம்பியான வெளுப்பு மாகும்
குண்டென்ற கோமூத்திர சிலாசத்து கோமூத்திர நிறமாகக் கட்டியாகும்
கண்டென்ற கனத்தில் பிறந்த சிலாசத்து கனகந்து செம்பரத்தப் பூ நிறந்த தாமே
நிறமான வெள்ளியுட சிலாசத்து தானும் நிசமான மயில் வயிற்றின் வெண்மை போலாம்
அறமான அழுக்கடைந்த கட்டியாகும் அதிய தாம்பிரந் தன்னிலுற் பவித்த
திறமான சிலாசத்து சொல்லக் கேளு சிறு நீல வண்ணமான கட்டியாகும்
முறமான முன் சொன்ன சிலாசத்து தானும் முறிந்து நோய் சலக்கட்டு நீருந்தானே

-- போகர் 7000 மூன்றாம் காண்டம்

| | | |
|---|---|---|
| *Velli silasathu* | - | White Variety |
| *Aya silasathu* | - | Cow's Urine Colour |
| *Kanaga silasathu* | - | Red Variety |
| *Tambira silasathu* | - | Light Blue Variety |

Other types are:

*Yema silasathu*

Blue and red *silasathu* are not found commonly. The *aya silasathu* is considered to be active. *Yema silasathu* and *thamira silasathu* are rare. *Yema silsathu* is used to treat *vatha* and *pitha* diseases.*Velli silasathu* is used in treatment of *kapha* diseases. *Aya silasathu* is used for treating tridosas and it is also used for rejuvenating treatment.

*Dhravis* are 4 types which comes under *komoothira silasathu.*

*1. Mangishadhravi*

*2. Asthidhravi*

*3. Annadhravi*

*4. Sangadhravi*

Life span of *komoorthira silasathu* is about 40 years.

**Classical methods to check the purity of *SILASATHU:***

Pure *silasathu* is obtained from persians.

A grain of *silasathu,* if put in water it will disintegrate completely and settle down at bottom and turn into red.

Pure *silasathu* has a Peculiar smell similar to cow's urine. It is like resin or can be hard but softens if a small portion is rubbed between the fingertips. It is lightweight and smooth to touch.

Pure *silasathu* will not smoke is put on a fire or charcoal ember. Silasathu is taken as a poppy seed size and heated with a little Ghee and this has to be given for the broken legs of hen. If it is pure means it will work within 2 hours. If it is not pure means it will work on next day.

Since it is a humic material it is bound to be contained with soil, rock particles and plant debris at the time of collection. So it needs to be purified before consumption.

**Purification of *Komoothira silasathu*:**

*Komoothira silasathu* is finally grind with milk, and keep it until it gets dry.

-Sarakku suthi muraigal,

**General Characters**

"கோமூத் நிரசிலா சத்தாற் குறுகியே
போமூத் திரமெரிவுட் புண்மேகம் -- நாமேவு
வெப்புதிர வெப்புமறும் வீழிமுனி விம்பந்
துப்புமஞ்சு மெல்லிதழாய் சொல்"

- *Gunapadam Thathu Jeeva Vaguppu*

It cures urinary infection, burning sensation and wounds in urinary tract, peramegam, apthus ulcer and hypertension.

"தீருமே கோமூத்திர சிலாசத் துத்தான்
செயமான வாதத்துக் குறுதியாகும்"

**Usages:**

**Traditional Uses:**

1. *Komoothira silasathu* is used for skin disorders. It is taken as 65 milligram and dissolved into hot water and it is used for diseases that affect tongue, cheeks.
2. Administration of *komoothira silasathu* with hot water or rose water droplets into ears and nose relieves ear pain and nasal disorders.
3. *Komoothira silasathu*, *kasthuri*, camphor are taken in equal amounts and mixed with betal juice. This has to be applied for headache externally.
4. *Komoothira silasathu* is mixed with honey and applied for tinnitus.
5. Chronic otitis and for other ear diseases, *Komoothira silasathu* is tied in a small cloth and have to kept inside ear.
6. 65 milligram of *Komoothira silasathu* with donkey's milk gives for Mania causes and in hemorrhagic cases.
7. 130 milligram *Komoothira silasathu* with ziziphus mauritiana (Indian jujube) juice or with honey or with milk and honey mixture given for dry cough twice a day.
8. *Komoothira silasathu* with cotton seed decoction, coriander decoction is given for *pitham,* stomach tumour.
9. To stop bleeding from piles, *Komoothira silasathu* is given by mixing with cow's ghee.
10. *Komoothira silasathu* is mixed with Betal decoction and it is used for elongated uvula and uterine haemorrhage.
11. It is used to treat urinary infections, urinary tract wounds, aphthous ulcers, hypertension, sexual disorders and give strength to heart and used as a service tonic and impotence. Cough, wheezing, intestinal disorders, infertility, *vatha* diseases and Inflammation.
12. Decoctions and juices of fruits like lemons and pineapple may be preserved for a long time by the addition of silajit.
13. In various parasitic skin diseases silajit may be used with much benefit.
14. silajit is used by the vaidyas in acute and chronic bronchitis.Benzoic acid and benzoates are administered especially for children .It promotes expectoration probably reflexly by causing irritation of the throat and stomach.

15. In pulmonary phthisis, silajit is very useful owing to its beneficial effect on the digestive and respiratory systems. It is most suitable for those cases where there is great thirst and burning sensation as in diabetic phthisis.
16. In sexual weakness it is generally administered with Aswagandha(withania somnifera)and other allied drugs.
17. In chronic gonorrhea and gleet, silajit is used with prepared oxides of tin,lead,silver etc.
18. Charaka says, there is hardly any curable diseases which cannot be controlled or cured with the aid of silajit.It is used by Kavirajas and Hakim's in a great variety of diseases.It is specially employed in genitourinary disease and in diabetes, gall stones, jaundice, enlarged spleen, fermentative dyspepsia, renal and bladder calculi, worms, digestive troubles, piles, anuria, hysteria, neurasthenia, epilepsy and insanity nervous diseases.

**Medicinal preparations of *komoothira silasathu*:**

**1.*Komoothirasilasathuparpam*:**

Dosage : 180 mg
Indication : *madhumegam*, All types of *Megam,* urinary infections, All types of Fever.
Reference : *Nam naatu vaithiyam - Pg.No: 171*

**2. *Silasathu Vathi kuligai:***

Dosage : 2 *kuligai,* 2 times after food per day.
Adjuvent : Milk,pomegranate juice.
Indication : *madhumegam,* anaemia, dropsy, fever, tuberculosis.
Reference : *Agasthiyar palathiratu. Pg.No: 62*

***3.Sathathy parpam:***

Dosage : 350-700 mg
Adjuvent : Honey,ghee
Indication : madhumegam, vathasoolai,peripheral neuropathy, peramegam, burning micturation.
Reference : *Agasthiyar paripooranam(*299). *Pg.No: 95*

***4.Komoothira silasathu parpam:***

Dosage : 200 mg

Adjuvent : Butter

Indication : *madhumegam, vatha* disease, leucorrhea.

Reference : *Ramadevar ennum vaithiya sinthamani* 7000. *Pg.No: 17*

***5.Komoothira silasathu chendooram:***

Dosage : 350 mg-700 mg

Adjuvent : Ghee, Butter

Indication : *madhumegam, vatha* disease, Anaemia.

Reference : *Nam Nattu vaithiyam -. Pg.No: 7*

## 3.1.2. GEO CHEMICAL ASPECT

### Shilajit (Asphaltum) :

Shilajit (Asphaltum) also known as mineral which is a pale brown to black is brown rock exudates found in many mountain ranges of the world, specially the Himalayas and hindukush ranges of the Indian subcontinent. Shilajit is a complex mixture of organic humic substances as well as plant and microbial metabolites which occur in the rock rhizophore substances identified in Shilajit include moisture, gums, albumunoids, resins, vegetable matter, benzoic acid, silica, minerals, vitamins and many other substances.

It is used for the last thousands of years as rejuvanator and as an adaptogen in traditional medicinal systems of many countries and has been attributed with miraculous healing properties. Recent reports have revealed that it has antioxidant, anti-inflammatory and anxiolytic activity. It has also demonstrated for spermatogenic and ovogenic effect in mature rats.

**Shilajit Odour:**

The common variety of shilajit has a typical odour resembling that of cow's urine and is branded as the "*gomutra shilajit*" in has a camphor like smell and is known as *karpur – gandha shilajit*. Extensive researches carried out on these two varities of Shilajit/

**Taste of Shilajit:**

Water soluble native shilajit has a slightly bitter, somewhat pungent, salty taste purified shilajit produces either acidic or alkaline taste depending on the quality and quantity of contained ingredients (Ghosal, 1989).

**Ealier Research on chemical constituents of Shilajit:**

Shilajit was earlier shown to be constituted of benzoic acid, hippuric acid, their salts and gums , albuminoids, traces of resins and fathy acids. The protein and mineral matters present in Shilajit produced on hydrolysis, glycine, protine, hydroxcyproline, Threonine and metal salts compiring of Ca, Na, K, fe sn, cu and si (chopra et al , 1958, shakir et al , 1964)

**Active ingredients in *SILASATHU:***

**Table No: 1 Organic Constituents of Asphaltum**

| S.No. | Organic Constituents | Crude Silajit | Pure Silajit |
|---|---|---|---|
| 1 | Moisture | 12.54% | 29.03% |
| 2 | Benzoic acid | 6.82% | 8.52% |
| 3 | Hippuric Acid | 5.53% | 6.13% |
| 4 | Fatty Acid | 2.01% | 1.63% |
| 5 | Resin and Waxy matter | 3.28% | 2.44% |
| 6 | Gums | 15.59% | 17.32% |
| 7 | Albuminoids | 19.61% | 16.12% |
| 8 | Vegetable matter, Sand, etc., | 28.52% | 2.15% |

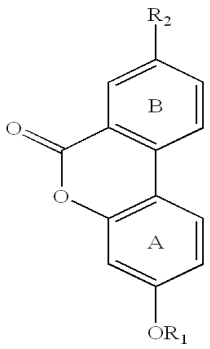
**Table No: 2 Mineral Constituents of Asphaltum**

| S.No. | The Mineral Constituents | Crude silajit | Pure silajit |
|---|---|---|---|
| 1 | Moisture | 12.54% | 29.03% |
| 2 | Loss of ignition | 64.58% | 52.03% |
| 3 | Ash | 22.88% | 18.34% |
| 4 | Silica (residue insoluble in HCl) | 4.60% | 2.69% |
| 5 | Iron ($Fe_2O_3$) | 0.51% | 0.67% |
| 6 | Alumina ($Al_2O_3$) | 2.26% | 2.61% |
| 7 | Lime(CaO) | 6.83% | 4.82% |
| 8 | Magnesia (MgO) | 1.29% | 1.20% |
| 9 | Potash ($K_2O$) | 4.60% | 3.81% |
| 10 | Sulphuric Acid ($SO_3$) | 0.64% | 0.97% |
| 11 | Sodium Chloride (NaCl) | 0.26% | 0.57% |
| 12 | Phosphoric acid ($P_2O_3$) | 0.28% | 0.24% |
| 13 | Nitrogen ($N_2$) | 3.64% | 3.36% |

The primary active ingredients in silasathu are fulvic acids, folic acid, dibenzo Alpha phones, humins, humic acids, trace minerals, vitamins A, B,C and D(citrines), phospholipids and polyphenol complexes terpenoids. Also, present are micro elements (cobalt, nickel, copper, zinc, iron, Chrome, iron, magnesium).

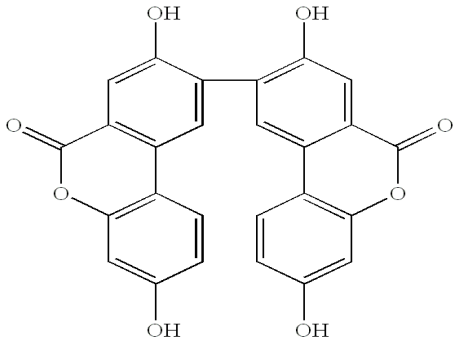
- Shilajit in Perspective

The purified shilajit of the invention includes (a) mono- or di-hydroxy or tetrameric dibenzo-α-pyrones (DBP) having the formulas shown below:

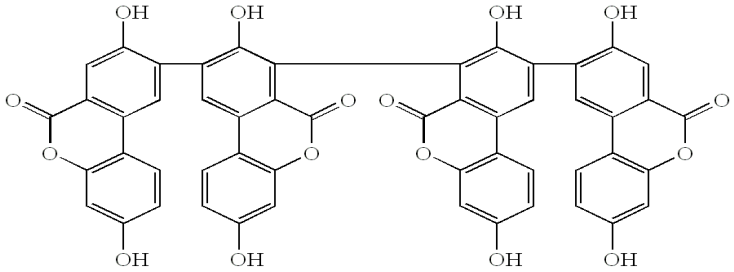



DBP

$R_1 = R_2 = H$
$R_1 = Me, R_2 = H$
$R_1 = H, R_2 = OH$

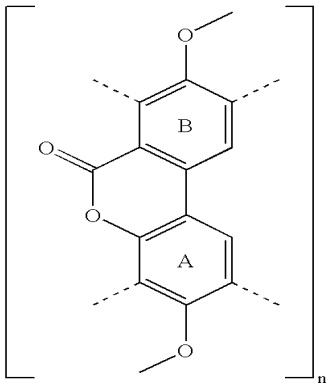



DIMERIC DBP & ITS HEMIQUINONE FORM




TETRAMERIC DBP & ITS HEMIQUINONE FORM

and (b) fulvic acids (FAs) which have repeat units having the formula:



The methanol soluble portion of the purified shilajit composition also includes 0.1-0.5% 3-OH dibenzo-α-pyrone; 0.3-1.5% 3,8-diOH dibenzo-α-pyrone; 0.001-0.1% eicosapentaenoic acid; 0.005-0.01% docosapentaenoic acid; 0.01-0.3% docosahexaenoic acid; 0.1-0.2% 2-hydroxyacetophenone; 0.01-0.2% 2,4-dihydroxyacetophenone and 0.05-0.3% α-lipoic acid.

The composition of the invention finds particular application in personal care, pharmaceutical and nutritional use formulations, suitably at a use level of 0.1 to 60% by weight of the composition, preferably about 0.2 to 10% in personal care formulations.

The purified shilajit compositions of the invention are obtained by an extraction procedure from native shilajit rock exdudate, as follows:

a) powdering native shilajit exdudate and dissolving it in water as solvent,
b) filtering the mixture to remove insoluble substances,
c) evaporating water from the filtrate to obtain a brown viscous residue,
d) extracting the residue with a hot organic solvent, e.g. methanol, to obtain both a soluble fraction which includes low $M_w$ bioactive phenolic compounds particularly oxygenated dibenzo-α-pyrones, and insoluble shilajit humic substances,
e) adding dilute aqueous NaOH to the insoluble shilajit humic portion to precipitate polymeric quinones,
f) acidifying the filtrate below a pH of about 3 to precipitate humic acids leaving a brown acidic solution of fulvic acids,
g) fractionating said acidic solution by passing it over activated carbon to provide a solution of low-to-medium $M_w$ fulvic acids,
a) passing the fulvic acid solution through a $H^+$ ion-exchange resin to concentrate the fulvic acids in solution, evaporating the solution, and
b) (j) combining the low-to-medium $M_w$ fulvic acids Mw 700-2000, with the low $M_w$ bioactive phenolic compounds in a suitable proportion, e.g. 9:1 by weight.

## 3.1.3. LATERAL RESEARCH

Effect of *silajit* on blood glucose and lipid profile in alloxan induces diabetic rats.

**Objective:**

To study the effect of silajit ( a herhomineral preparation) on blood glucose and lipids profile in euglycemic and alloxan induced diabetic rats and its effects on the above parameters in combination with conventional antidiabetic drugs.

**Materials and methods:**

Diabetes was induced in Albino rats by administration of a single dose of alloxan monohydrate 5 %(125 mg/ kg). Effects of three different doses of silajit (50,100&200 mg/kg/day orally), alone for 4 weeks and combination silajit (100 mg/kg/day, orally).With either glibenclamide(5 mg/kg/day, orally) or metformin (0.5 g/kg/day, orally) for 4 weeks was studied on blood glucose and lipid profile.

**Results:**

In Diabetic rats, all the three doses of silajit produced a significant reduction in blood glucose levels and produced beneficial effects on lipid profile. The maximum effect was observed with the 100 mg/kg/day dose of silajit.

**Conclusion:**

Silajit is effective in controlling blood glucose levels and improve the lipid profile.

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20, July, 2004.

## 3.2. Terminalia chebula.*Retz* (*KADUKKAI*)

### 3.2.1. GUNAPADAM ASPECT

**Synonyms:**

*Amutham, Ammai, Arithaki, Arabi, Aviyatha, Amritha, Aparanam, Anthan, Anganam, Amalai, Alliyan, Resaki, Yemavathi zyavi, Himavathi, Kadu, Kaayastha, Siyurutham, syuraki, Sirotam, Siva, Sethaki, Seya, Divya, Devi, Nandhini, Nechi, Pathiyam, Megam, Boothana, Paariyam, Vayatharam, Rarikkai, Vanadurki, Vijayavedhan, Rohini, Yeevaniga, Jeevanthi, Jeevapriya, Jeevya, Jeya.*

**Vernacular names:**

| | |
|---|---|
| Tamil | : *Ammai, Amutham, Aritaki, Pethiyam, Varikkai* |
| Telugu | : *Karakkaya* |
| Urdu | : *Halela* |
| Bengali | : *Haritaki* |
| Hindi | : *Harre, Harad, Harzar* |
| Kannada | : *Alalekai* |
| Malayalam | : *Katukka* |
| Marathi | : *Hirdi, Haritaki, Harda, Hireda* |
| Punjabi | : *Hakeka, Harar* |
| Sanskrit | : *Maritaki, Abhaya, Kayastha, Siva, Pathya* |
| Assamese | : *shilikha* |

**Parts used:**

Mature fruit and immature fruit.

**Organoleptic character:**

| | |
|---|---|
| Taste | : Mainly-Astringent, slightly sweet and sore, pungent, bitter. |
| Potency | : Hot |
| Biological Transformation | : sweet |

**Action:**

Cardiac stimulant

Tonic

**General Characters:**

"தாடை கழுத்தக்கி தாலு குறியிவிடப்
பீடை சிலிபதமுற் பேதிமுடம் - ஆடையெட்டாத்
தூலமிடி புண்வாத சோணிகா மாலையிரண்
டாலமிடி போம்வரிக்கா யால்."

- Gunapadam Mooligai Vaguppu
- Page No: 207

**General characters:**

**Kadukkai:**

It cures excessive urination, aguseia, heart diseases, hypertension, traumatic wounds, lip disease, eye disease, cough, jaundice, worm infestation, gastric disorders, leprosy, peripheral neuropathy, respiratory disorder, head disease, anaemia,bronchial asthma, hoarseness of voice, excessive thirst, impotency, liver diseases, leucorrhea, ascites,dryness of tongue, tongue diseases, vatha diseases, chest diseases, urinary disorders, piles, venereal diseases, abdominal disorders, hiccup, vitiligo, fever due to poisoning, tuberculosis, constipation, indigestion, cough, internal body heat, kapha vatha diseases, skin disease, anal fistula, cancer, stomach tumour and apthus ulcer.

**Usages:**

**Traditional uses:**

1. chebulic myrobalan cures aguseia
2. Decoction of Chebulic myrobalan cures eye diseases, polyuria. powder of Chebulic myrobalan used as a tooth powder to reduce the pain of gums, bleeding gums.
3. chebulic myrobalan powder inhalation cures sinusitis.
4. whole plant of Chebulic myrobalan grinds with milk is given for eczema,leprosy.
5. The fruit is mild laxative,stomachic,tonic,alterative,antispasmodic.It is useful in opthalmia, hemorrhoids, dental caries, bleeding gums, ulcers oral cavity.
6. The paste of Chebulic myrobalan with water is found to be anti inflammatory, analgesic and having purifying and healing capacity for wounds.
7. chebulic myrobalan decoctions is used as gargle in oral ulcers, sore throat.
8. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinectic agent and mild laxative.

9. The powder of Chebulic myrobalan fruits has been used in chronic diarrhoea. It is used in nervous irritability.
10. chebulic myrobalan is adjuvent in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as in asthma. Also it is useful in renal calculi, dysurea, retention of urine and skin disorders with discharges like allergies, urticaria and other erythematous disorders.
11. If chebulic myrobalan is regularly taken in every morning for a period of one year grey hair turns black and wrinkles of skin disappear
12. outer skin of Chebulic myrobalan, Zyzygium Aromaticum taken about 4 gm and made into decoction by boiling it for 10 minutes and filtered and it is used for dysentery.

**Other preparations in *kadukkai*:**

***1. Muthakasu kulambu:***

Dosage : 500 mg
Indication : *madhumegam,* urinary infection
Reference : *SarbendraVaithiya Rathinavali* Pg. No.: 21

***2. Keelanelli paste:***

Dosage : 1 gm
Indication : *madhumegam*
Reference : *Sarbendra Vaithiya Rathinavali* Pg. No.: 23

***3. Kadalurinchiyathi pattai chooranam:***

Dosage : 2-5 gm
Indication : *madhumegam, piramegam*
Reference : *Sarbendra Vaithiya Rathinavali.* Pg. No. : 70

***4. Avarai vithai chooranam:***

Dosage : 488 mg
Indication : *madhumegam, athimoothiram*
Reference : *Mega Nivarana Bodhini* Pg. No: 49

***5. Thettran vithai kudineer:***

Dosage : 35 ml per day, 6 times

Adjuvent : Butter milk

Indication : *madhumegam, soolai*

Reference : *Gunapadam Mooligai Vaguppu* Pg. No.:551

***6. Vithatti chooranam:***

Dosage : 51g (1 kalanchu)

Indication : 21 types of *piramegam,* diabetes mellitus (*madhumegam*), spermatzuria, premature ejaculation, gonococcal infection diseases.

Reference : *Anubooga Vathiya Navaneetham* Pg. No:126

## 3.2.2. BOTANICAL ASPECT

Botanical name :*Terminalia chebula. Retz*

**Classification:**

| | |
|---|---|
| Kingdom | : plant kingdom |
| Division | : Angiosperms |
| Class | : Dicotyledons |
| Subclass | : Polypetalae |
| Series | : Calciflorae |
| Order | : Myrtales |
| Family | : Combretaceae |
| Genus | : Terminalia |
| Species | : Chebula |

**Morphology:**

**Distribution:**

Throughout India in deciduous forests and on hill slopes upto 200m, also cultivated in plains.A tree small to medium in size, the average height being 5-5 metres. It's bark is usually light brown to black, coming off in thin strips or flakes, exposing the fresh surface of a differ colour underneath the older bark.

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**Leaves:**

Leaf buds, branchlets and youngest leaves with soft, shining, generally rust coloured hairs, leaves 7-20 cm, by 4-8 cm glabrous or nearly so when mature come or not clustered, distant alternate or sub-opposite, elliptic oblong, rounded or cordate at base, penninerved, second nerves 6-8 pairs, arching, prominent.

**Petioles:**

2-5 cm long, pubescent, usually with 2 glands near the top.

**Bracts:**

It is existing in the flowers subulate or lanceolate Hairy. Conspicuous among the buds but soon deciduous.

**Calyx:**

Capitulate, 3 mm long constricted about the ovary flat the base expanding a little towards the mouth glabrous outside, hairy inside teeth short sometimes obscure.

**Bark:**

6 mm, dark brown with many generally Shallow vertical cracks exfoliating in axales.

**Wood:**

very hard, brownish grey with a greenish yellowish fringe, with a irregular small dark purple heart wood, close grained

-India medicinal plants p.no. 1020-1021.

**Flowers:**

Flowers are hermaphrodite, 4 mm across sessile, dull white or yellow with an offensive smell.

**Style:**

Stipulate ending in a small stigma.

**Stamens:**

10 in two series arising from the calyx-tubes.

**Fruit :**

Fruit is simple and drupaceous. It is pendulous, 2 to 4 cm long and is mostly egg shaped. Oblong or elongate, elliposidal 15 to 25 me, wide at the broadest part. It is glabrous and obscurely or faintly 5 ridged that are 2 to 3 mm wide and 1 to 2 mm thick. The surface colour varies from light yellowish brown to a nearly uniform brown with yellowish markings or patches here and there. In some fruits, the basal portion is narrower and some what elongate or tapering. When the fruit is dry, it is bony hard, very thick and obscurely angled. Seeds are hard and pale yellow in colour.

The cross section of the dried fruit shows mainly two parts.

pericarp

seed

**Pericarp:**

Pericarp comprises of the rind, which is composed of the very thin and closely adhering skin or epicarp and the medical, and a hard or stony endocarp. On account of presence of ridges and furrows on the the surface of the fruit the thickness of the rind epicarp and mesocarp varies from 2 to 4 mm at the furrows to 4 to 6 mm

at the ridges. The relative thickness of the rind with that of the bony endocarp often varies in different fruits. The colour of the mesocarp is greyish yellow and that of the endocarp is pale yellow.

**Seed:**

The seed has a thin brownish skin or testa and the embryo within whitish.

**Collection of fruits:**

The fruits are to be collected in first half of January from the ground as soon as they have fallen or the matured fruits are to be collected during January to April by shaking the trees and then they are dried in shade and store.

**Chemical constituents:**

Terminalia chebula contains chebulin MP 249 from flowers, a purgative glycoside of an anthraquinone derivative isolated, a tannin terchebin from fruits.

-compendium of Indian medicinal plants volume 1

Anthraquinine, glycoside, chebulinic acid, chebulagic acid, tannin acid, tercgebulin, tetrachebulin, vitamin c, arachidic, behenic, linoleic, oleic, palmitic and stearic acids(fruit kernels), chebulin (flowers)2 -alpha hydroxy minor medic acid, maslinic acid and 2- alpha- hydroxy ursolic acid (leaves).

**Phytochemical aspect:**

| | | |
|---|---|---|
| Flowers | : | chebulin |
| Fruit | : | phenolic compounds, punikala, terflavin A, Ellagin, |
| | | tannin terchebulin |
| | | Terchebin |
| | | Gallic acid |
| Kernels | : | Fatty oil |
| Tree | : | Gum |
| Myrobalan | : | 30-32% Tannic acid |
| | | Mucilage |
| | | Chebulinic acid |
| | | Brownish yellow colouring matter |

## 3.2.3. LATERAL RESEARCH

Anti-diabetic activity on Ethanolic extracts of fruits Terminalia chebula. Alloxan induced diabetic rats.

**Abstract:**

The present study intended to evaluate the beneficial effects of ethanolic extracts of terminalia chebula fruits(EETC) by using alloxan monohydrate induced diabetic control by using wistor Albino rats. The toxicity study was performed on aliquot drones of EETC (100_500 mg /kg bwt) and predetermined the LD 50 value on 30 days evaluation; also the behavioral changes symptoms and mortality have been checked. The EETC show the toxicity up to(500 mg/kg btw). The effect of EETC was compared with the glibenclamide (600 mg/ kg btw) that is often used as a standard drug and the anti_diabetic activity has been conducted for 30 days. After the completion of the study the animals were dissected through cervical and and collected the blood serum and pancreas. The collected samples were performed under parameters like biochemical and antioxidant enzymes related to diabetes such as weight variation, blood glucose, plasma insulin, serum and liver protein serum cholesterol, serum and liver protein, serum and liver phospholipids, SGOT(serum glutamate oxaloacetic transaminase), ACP( acid phosphatase),ALP( Alkaline phosphatase),GSH(Gluta reductase),GPT( glutamate pyruvate transaminase),GPX ( glutathione peroxidase), CAT( catalyst) and histopathologic and sections of the pancreas. The above parameters calculated and showed that the significance at $P<0.001$ to 0.05. The histopathological changes caused after induction of alloxan show the granular cytoplasm dilation, shrunken nuclei and inflammation, which were reduced after the treatment of EETC (200 mg kg b.wt). Excess proliferation of epithelium in the pancreas was observed in Diabetic rats which was reduced after administration of EETC having the pharmacological action against the diabetic condition even the mechanism of the action is unknown, also it can be used to further molecular compound analysis and define the chemical action.

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American Journal of Drug Discovery & Development 2(3) 135 - 142,2012

## 3.3. Phyllanthus emblica dried(*Nelli mulli)*

### 3.3.1. GUNAPADAM ASPECT

**Synonyms:**

*Amalukam, Soolakam, Ambal, Amarigam, Thatharis, Korangam, Miruthubala, Meerthinthu.*

-Gunapam-Mooligai Vaguppu

-P. No.: 620

"அதுவென்ற வந்திகோர மென்றும் பேரு சன்னதமா மயலநதி காரனென்னும் பேரு
யதுவென்ற வாமலக்கி யென்றும் பேரு அறுவான அமுர்த மென்னும் பேரு
மதுவென்ற மாமலக்கா மென்னும் பேரு மகத்தான மாலக மென்னும் பேரு
வதுவென்ற லவுடாஞ்சி துத்திரிக மென்னும் அருளினோம் நெல்லிக்காய் அதற்கும் பேரே"

பொழிப்புரை :

அந்திகோரம், ஆமலக்கி, அமுர்தம், துத்திரிகம், ஆமலகம்

--தேரையர் காப்பியம்

பக்கம் 105

**Vernacular names:**

| | | |
|---|---|---|
| English | : | Emblic Myrobalan Tree |
| Arabic | : | *Ambily, Amlaj* |
| Assameese | : | *Amlaki, Amluki, Sohmyralin, Amlaku, Amalaki, Amulakh, Amlakhi,* |
| Bengal | : | *Amla, Ambolatti, Amlaki, Amlati* |
| Hindi | : | *Amalaci, Amla, Amlika, Anola, Anli, Anvula, Anvurah, Aungra, Aunra, Daula* |
| Malayalam | : | *Amalakam, Nelli* |
| Marathi | : | *Anvala, Aonli, Avala, Arola, Bhuiawali* |
| Sanskrit | : | *Adhiphala, Akara, Amalaki, Amamalakam, Anlika, Amruphala, Amrita, Amritaphala, Dhatri, Dhatriphala, Jatiphala, Shanta, Shiva, Shriphali, Tishya, Tishyaphala, Vrishya, Vayastha* |
| Telugu | : | *Amalakamu, Amalaki, Nelli, Pullayusprika, Uirika, Usirikaya* |
| Urdu | : | *Anwala* |

**Parts used:**

Leaf, Bark, Root, Seed, Fruit

**Organoleptic character:**

| | |
|---|---|
| Taste | : Astringent, sweet, sour |
| Potency | : Neutral |
| Biological transformation | : Sweet |

**Action:**

Fruit

Diuretic, Refrigerant, Laxative

Flower

Refrigerant, Laxative

Leaves, bark, dried fruit-

Astringent

**General Characters:**

"நெல்லிக்காய் பேர்தனையே நிகழ்த்தக்கேளு தேயமாஞ் சபலமாஞ் சிதுபலமாகுந்
தல்லிக் காந்திரிபல மாந் சலமுமாகுந் சமான வாமங் கதறிசிய பலமாகும்
வில்லிக்காய் சிறிபலமா மேவசமுமாகும் விளங்கியதோர் பஞ்சசாவறு' பலமாரும்
நெல்லிக்காய் சிறிதோ' பணியமிகுஞ் செப்பியதோர் நெல்லிக்காய் பேகுமாமே"

--போகர் நிகண்டு
பக்கம் - 284

It gives good taste to the tongue.It cures internal excessive body heat, rickets, hypertension,menorrhagia,psychiatric disorder, oliguria, vomiting, leucorrhea, penieal vesicle. If apply the paste of nellimulli to the hair it can be gives cooling effect of eye.

**Types:**

| **Tamil Name** | **English Name** | **Botanical Name** |
|---|---|---|
| 1. Nilla Nelli | Lowly Nelly | Pyllanthus madraspatensis |
| 2. Thoopu Nelli | Garden Nelly | Emblica officinalis |
| 3. Kattu Nelli | The same as Nelly | Emblica officinalis |
| 4. Karu Nelli | Black honey shrub | Phyllanthus reticulates |
| 5. Kodi Nelli | an unknown variety | |
| 6. seemainelli | 1. Barbadoes cherry | Eugenia uniflora |
| | 2. Common ovate leaved barbadoes | |

| | | |
|---|---|---|
| | Cherry – Malpeghia coccigera | |
| 7. Arunelli | Country star goose berry | Phyllanthus distichus |
| 8. Sennelli | a red variety of nelli | |
| 9. Mellanelli | Same as Karunelli | |
| 10. Kellanelli | Indian annual phyllanthus | Phyllanthus niruri |
| 11. Neernelli | Same as karunelli | |
| 12. Nilanelli | Same as Arunelli | |
| 13. Vennelli | whitenelli | Phyllanthus pendulous |

-Tamil English dictionary, T. V. Sambasivam pillai volume-4

**Traditional uses:**

1. Prepare thuvaiyal using phyllanthus emblica and eat along with regular food. This will remove tasteless and vomiting sensation.
2. Make decoction out of dried phyllanthus emblica fruit and drink. This will remove excessive thirst, giddiness and vomiting sensation..
3. Boil leaves of phyllanthus emblica in water.Gargle with water. Mouth ulcers will be cured.
4. Mix15ml each of the juice of phyllanthus emblica, lemon, honey and drink only in the morning. This will cure Diabetes mellitus.
5. Grind the tender leaves of phyllanthus emblica and mix with buttermilk and drink this can cure dysentery.
6. Make decoction out of dried phyllanthus emblica. To this add milk and sugar and drink. This will cure internal heat vomiting and also pustles in the penis.
7. Take 20 gram each of dried phyllanthus emblica and green gram and add one litre of water boil till the volume is reduced to 200 ml. 1 100 ml in the morning and remaining in the evening. This will cure blood pressure associated with giddiness.
8. Take half litre is of the juice of phyllanthus emblica, Altementhera sessions (ponnaangkanni), Aloe Vera, Amaranthus polygamus(chirukeerai) and milk. Take 2litres of coconut water and 1.5 litres of gingelly oil and mix them well. To this add 15 grams each of the following: licorice, cardamom, costus spiceosus, curcuma zedoraia, curcuma aromatica, nutmeg, maze, dried ginger, pepper, long pepper, terminalia chebula and bellerica. Boil to consistency.

Use this oil for taking oil bath twice a week. This will cure jaundice Eye diseases, night blindness, dandruff, falling of hair etc.

- Medicinal plants of India- Volume II.

9. The fruit is useful in burning sensation, vomiting, urinary discharges, thirst, leprosy, constipation, inflammations, crisscross,piles, anaemia, stangury, very, anuria, poisoning ophthalmia incident blindness.
10. The seed is useful in biliousness asthma, bronchitis, leucorrhoea, vomiting

- Indian medicinal plants volume 3

11. Acute bacillary dysentery may be arrested by drinking a sharbat of Amla with lemon juice.
12. Triphala consisting of equal parts of powdered emblic myrobalan, belleric myrobalan used as laxative and in headache, biliousness, dyspepsia, constipation, piles, and large liver and acscites.
13. The oxidation from insistence on the fruit is used as external application for inflammation of eye.
14. Dried fruit is a detergent and is used as shampoo for the hair.
15. A Fixed oil extracted from the fruits is reported to have the property of promoting hair growth.

- The wealth of India
volume 3

16. If Nellikai is taken internally gives younger appearance.
17. Nellikai thuvaiyal can cure anorexia, vomiting.
18. Decoction of Nellikai cures fainting, thirst, nausea.
19. Taste of tender leaves mixed with buttermilk and is given for dysentery. Decoction of Nellivatral mixture with sugar and milk can be given internally for excessive 130 heat, wounds in male genitalia and vomiting.

-Gunapadam mooligaivaguppu
P. No. 392

**Medicinal Preparations of *Nellimulli*:**

***1.Aya chendooram:***

Dosage :10gm-50gm

Indication :*Madhumegam*

Reference :*kannusamiyam ennum vaithiya Rogam. Pg.No: 50*

***2.Maruthampattai Kasayam:***

Dosage :30ml -60ml

Indication :*madhumegam*

Reference :*sarabendra vaithiya rathina vali. Pg.No: 22*

***3.Nelliyathi kasayam:***

Dosage :30 ml

Adjuvent :Honey

Indication :diabetes Mellitus, urinary infections *Pg.No: 127*

***4.Avaraivithai chooranam***:

Dosage :5gm - 10gm

Adjuvent :Buffalo curd

Indication :diabetes mellitus

Reference :*sarabendra vaithiya rathna vali. Pg.No: 185*

***5.Thirikadugu orundai:***

Dosage : 6.022gm

Adjuvent :.Honey,daily two times a day

Indication : diabetes mellitus

Reference : *sarabendra vaithiya rathna vali. Pg.No: 96*

***6.oorkuruvi ilagam:***

Dosage :4.16gm

Indication : *peramegam*, general weakness, spermaturia, *Pitham*, burning micturation, cough,tuberculosis,diabetes Mellitus.

Reference :*Anubooga vaithiya navaneetham. Part 2. Pg.No: 75*

***7.Abraga chendooram:***

Dosage : 260 – 340 mg

Adjuvent :Honey,ghee,butter

Indication :21 types of megan, 21 types of madhumegam, all types of vatha, pitha disease.

Reference :*Anubooga vaithiya navaneetham. Part 2. Pg.No: 106*

## 3.3.2. BOTANICAL ASPECT

**Botanical name** : Phyllanthus emblica

**Classification:**

| | |
|---|---|
| Kingdom | : Plantae |
| Division | : Angiospermae |
| Class | : Dicotyledonae |
| Order | : Geraniales |
| Family | : Euphorbiaceae |
| Genus | : Phyllanthus |
| Species | : Emblica |

**Morphology:**

**Habit**:

It is a small to medium sized deciduous tree, reaching 8 to 18 m in height with the crooked trunk and spreading branches. Its bark is usually light brown to black coming of in thin strips of flakes, exposing the fresh surface of a different colour underneath the older bark. The average girth of the main stem is 70 in most cases the main trunk is divided into 2 - 7 scaffolds very rear to the base.

**Leaves**:

Leaves simple, subsessile closely set along the branchlets, distichous, light green having the appearance of pinnate leaves. The leaves develop after the fruit set.

**Flowers**:

Flowers unisexual, pale green, 4 to 5 millimetre in length, born in leaf-axils in clusters of 6 - 10.

**Staminate Flowers**:

Tubular at the base having a very small stalk, gamosepalous, having six lobes at the top. stamens 1 - 3 polyandrous filaments 2 mm long.

**Distillate Flowers**:

Disc Cup shaped edge toothed having a gamopetalous Corolla arid to branched style, ovary 3 celled.

**Fruit**:

Fleshy globose, ½ - 3/4 inx diameter with 6 vertical furrows, pale yellow, sometimes it is 3 celled and 6 seeded.

- 1. Indian trees
pg. no. 570
- 2. Indian Medicinal plants vol. 4
pg. no. 256

**Macroscopic description of dried fruit:**

It consists of curled pieces of pericarp of dried fruit occurring either as separated single segment 1_2 centimetre long or united segments. Grey to black pieces showing a broad highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface. External surface shows a few white specks occasionally some pieces show a portion of stony testa. Texture is rough, cartilaginous and tough.

**Microscopic description of dried fruit:**

Transverse section of fruit shows epicarp consisting of a single layer epidermis cells appearing tubular and polygonal in surfaceview, cutide present; mesocarp cells tangentially elongated,parenchymatous and crushed, differentiate roughly into a peripheral 8_9 layers of tangentially elongated smaller cells, rest consisting of mostly isodiametric larger cells.

**Phytochemistry:**

**Fruit:**

Rich source of vitamin C, phyllembin from fruit pulp is identified as ethyl gallate. Fruit also contains curcurminoides. the edible fruit tissue contains protein concentration three fold and ascorbic acid concentration 160 fold compared to that of the Apple. The fruit also contains considerably higher concentration of most minerals and amino acids than Apple. Fruit juice contains nearly 20 times as much as Vitamin C as orange juice and a single fruit is equal in antiscorbutic value of one or two oranges.

| | |
|---|---|
| Glutamic acid | -29.6% |
| Propline | -14.6% |
| Aspartic acid | -8.1% |
| Alanine | -5.4% |
| Lysine | -5.3% |

The dried pulpy portion of the fruit contains.

| | |
|---|---|
| Gallic acid | -1.32% |
| Tannin | -36.1% |
| Sugar | -36.1% |
| Gum | -13.75% |
| Albumin | -13.08% |
| Crude cellulose | -17.08% |
| Mineral matter | -4.5% |
| Moisture | -3.83% |

The fresh fruit pulp contains

| | |
|---|---|
| Moisture | -81.2% |
| Protein | -0.5% |
| Fat | -0.1% |
| Mineral matter | -0.7% |
| Fibre | -3.4% |
| Carbohydrates | -14.1% |
| Ca | -0.05% |
| P | -0.02% |
| Fe | -1.2mg/100g |
| Micotinic acid | -0.2mg/100g |
| Vitamin C | -600mg/100g |

Superoxide dismutase -482.14 units/g

Apart from L-Ascorbic acid, emblica fruits also contain 'ascorbigen', an indole containing descriptive od L-ascorbic acid (Vitamin C). Ascorbigen and its derivative N- Methyl ascorbigen representa new class immunomodulators.

**Fruit ash:**

| | | |
|---|---|---|
| Chromium | - | 2.5 ppm |
| Zinc | - | 4 ppm |
| Copper | - | 3 ppm |
| Fruits and leaves | - | Tannins, polyphenolic compounds |
| | - | Trigauopyl glucose, Terchebin, Corilagin, Ellagic, Phyllenbic acids. Alkaloids - Phyllantidine and phyllantine. |

| | | |
|---|---|---|
| Leaves and stem | - | Lupeol and Beta sistosterol. |
| Seeds | - | Linnoleic acid and it closely resembles linseed oil. |
| Roots | - | Ellagic acid and Lupeol. |

**Chemical constituents:**

These fruits are reputed to contain high amount of ascorbic acid (Vitamin C) and have bitter taste that may derive from a high density of ellagitannins, such as emblicanin A(37%), emblicanin B(33%), punigluconim (12%) and pedunculagin (14%). Amla also contains punicafolin and phyllanemblinin A phyllanemblin other polyphonic, such as flavonoids KaempFerol, ellegic acid, Gallic acid.

## 3.3.3. LATERAL RESEARCH

Antidiabetic and antioxidant potential of Emblica officinalis Gaertn. Leaves extract in streptozotocin induces type -2 diabetes mellitus (T2DM) rats.

**Abstract:**

In traditional Indian Medicine all parts of Emblica officinalis Gaertn. Plant including the fruit seed leaves fruit bark and flowers are used in various herbal preparation for the treatment of diabetes mellitus.

**Aim:**

To evaluate the effects of hypoglycemic effects of the hydro methanolic(20:80) extract of leaves of emblica officinalis in streptozotoxin induced diabetic rats.

**Author information:**

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# 3.4. Terminalia Bellarica *(Thandrikkai)*

## 3.4.1. GUNAPADAM ASPECT

**Synonyms:**

*Aksham, Akhandam, Amudham, Ambhalathi, Aaraamam, Erikatpalam, Kandhakatpalam, kandhukan, sakadham, Thabamari, vandhiyam, vithiyam, Bhoodhavasagam, Thanikai, Thrilingam, vipeethagam.*

**Vernacular names :**

| | |
|---|---|
| English | :Belleric myrobalan, Beddanut tree. |
| Hindi | :*Bhaira,Bahera.* |
| Kannada | :*vibhita,Taremera,Tharo,Taarekaayi Mara, Thani, vibhitaka.* |
| Malayalam | :*Thani, Thannikka, Adamarutha, Thanni.* |
| Marathi | :*kalidruma,vehala,Behada,Thannymathan,Thaanni.* |
| Sanskrit | :*Bibhitakah, Bahuvirya, vibhitakah, kasaghnah, karshah.* |
| Telugu | :*Bhutavasama,vibhitakamu,Tandrachettu.* |
| Urdu | *:Behera.* |
| Gujarathi | :*Bhaira,Bahera.* |

**Organoleptic characters:**

| | |
|---|---|
| Taste | : Astringent |
| Potency | : Hot |
| Biological transformation | :Sweet |

**Action :**

Astringent

Expectorant

Laxative

Tonic.

**General Characters:**

“சிலந்திவிடம் காமியப்புண் சீழான மேகங்
கலந்துவரும் வாதபித்தங் காலோ- டலர்ந்துடலில்
ஊன்றிக்காய் வெப்ப முதிரபித் துங்கரக்குந்
தான்றிக்காய் கையிலெடுத்தால்

ஆணிப்பொன் மேனிக் கழகும் ஒளியுமிகும்
கோணிக்ககொள் வாதபித்தக் கொள்கைபோம் தானிக்காய்
கொண்டவர்க்கு மேகமறும் கூறா அனற்றணியும்
கண்டவர்க்கு வாதம் போம் காண்.”

- குணப்பாடம் மூலிகை வகுப்பு

It cures poison of spider bite, wounds in the penis, leucorhea, hypertension, disease due to excessive vatham and Pitham. It gives brightness and beauty to body as well as it can neutralise Tridosas.

**Usages**:

**Traditional uses:**

1. For tooth ache the outer covering of *Thandrikai* and make it as a fine powder and mix it with same amount of sugar can be given 4 grams per day with lukewarm water.
2. Powder of *Thandrikai* mixed with honey can be administered for chickenpox.
3. Decoction should be prepared from 170 ml of water with 17.5 grams of skin of *Thandrikai* then decoction mixed with 20 gram of honey given for bronchial asthma.
4. Powder of *Thandrikai* mixed with equal amount of white sugar and honey is given for gram daily for the improvement of vision.
5. Seed of *Thandrikai* is rubbed and the paste is applied over the wounds.
6. Powder of *Thandrikai*, mixed with equal amount of rock salt and powder of long pepper then the above mixture is grinded with buttermilk and given for hoarseness of voice.
7. Decoction of *kadukkai*, *nellikai*, *Thandrikai* is used for wound washing.

- Gunapadam mooligai vaguppu.

**Medicinal Preparations of *Thandrikai*:**

***1.Thetrankottai leghyim:***

| | |
|---|---|
| Dosage | : 2-5 gm 2 times per day |
| Adjuvent | : *Naga parpam*(65 mg) |
| Indication | : *madhumegam.* |
| Reference | : *Anubooga vaithiya navanitham* part 2 |

***2.Maha vinthathi kuligai:***

| | |
|---|---|
| Dosage | :1 -2 kuligai |
| Indication | : *madhumegam, piramegam, Thanthumegam*, 9 types of *Moolam*, 6 types of *powthiram.* |
| Reference | :*Anubooga vaithiya navanitham* part 2. |

***3. Visuvasi chooranam:***

Dosage :2 gms twice a day, after food

Indication :*madhumegam*

Reference :*yugi muni vaithiya Kaviyam* p. No-263.

***4. Sooriya mantha kudineer:***

Preparation :*Aavaripattai, Thetanvithai, kadalalinjil patti, kadukkai, seeragam* are powdered and add 8 part of water to the powder and boil to make 1 part of decoction.

Dosage :100 ml two times a day before food.

Indication :*madhumegam*

Reference :*yugimuni vaithiya kaaviyam* p. No-664.

***5.Aya cheendoram:***

Dosage :200 -400 mg.

Adjuvent :Ghee,honey

Indication :*madhumegam*, Anaemia, body heat.

Reference :*pathartha guna vilakam, Thathu-jeeva Vargam.*

***6. Santhanathy ennai:***

Dosage :Externally used

Indication :*neerelivu,kallaidupu*,Tuberculosis,Asthma,urinary tract infection.

Reference :*Agasthiyar* 2000 P. No-13.

## 3.4.2. BOTANICAL ASPECT

**Botanical Name** : Terminalia Bellarica

**Classification:**

Kingdom : Plantae

Subkingdom : Tracheobioneta

Division : Magnoliophyta

Class : Mangnoliopsida

Subclass : Rosidae

Order : Myrtales

Family : Combretaceae

Genus : Terminalia

Species : Bellarica

**Morphology:**

A large tree, grows upto 20-25 meters high, rust coloured pubescence on young branchlets.

**Leaves:**

Simple, opposite alternate clustered at the tip of the branchlets, estipulate, petiole 15-80 mm, stout slightly grooned above, glabrous, lamina 9-35 * 5-16 cm. Absolute, elliptic or obovate-elliptic; base obliquely cuneate, attenuate or acute; apex intensely accuminate, margin entire, both surface pubescent when young, glabrous at maturity, coriaceous, eglandular, lateral nerves 7-10 pairs, innate, prominent, intercostae reticulate.

**Flowers:**

Bisexual, greenish yellow , 5-6 mm across axillary spikes, peduncle puberoulous, bracteoles 0.5-2mm long, linear_lanceolate, caducous, calyx tube 2-2.5*1-3.2 mm, rusty pubescent, constricted above the ovary, lobes 5, cream, triangular, tomentose, disc 5 lobed, petals absent, ovary 1.5mm inferior, tomentatse, 1-celled, couples 2-3, pendulous, style 4mm, subtulate, stigma small.

**Fruit:**

A drupe 2-2.5*1.8 cm, obovoid, obscurely 5-ridged, yellowish-Brown, hoved, not winged, softly tomentose.

**Seed:**

One, ellipsoid.

**Growing season and type:**

1. Pale yellow with an offensive order in axillary spikes.
2. They Blossom in the month of May.
3. Fruits ovoid grey. Fruits are 1.5 to 2.5 cm in diameter.
4. The kernels are sweet, but narcotic.

-www.jnkuv_aromedicinal plants.in

**Phytochemistry:**

| | | |
|---|---|---|
| **Fruits** | **:** | chebulagic acid, ellagic acid and its ethyl ester, fructose, galactose, glucose and its galloyl derivative, mannitol and rhamnose and beta sitosterol. |
| **Bark** | **:** | Chebulagic acid and ellagic acid |
| **Seed coat** | **:** | Gallic acid. |

## 3.4.3. LATERAL RESEARCH

**Antidiabetic and antioxidant activity of terminalia bellerica: Roxb**

**Abstract:**

Effect of continuous administration of dried 75 % methanolic extract of fruits of terminalia bellerica(combretacease) suspended in water was studied in alloxan induced hyperglycemia and antioxidant defence mechanism in rats. Terminalia bellerica prevented alloxan induced hyperglycemia significantly from 6thof Administration and near was 54 %reduction on 12th day. Oxidative stress produced by alloxan was found to be significantly lowered by the administration of terminalia bellerica extract. This was evident from a significant decrease in thiobarbituric acid reactive substances.

Conjugated dienes and hydroperoxide in blood and liver respectively, similarly, decreased glutathione level produced by alloxan was increased by the administration of the extract in blood and liver. However the increase was not significant. Superoxide dismutase which was decreased by alloxan were significantly increased from 9th day in blood and liver of drug treated group. Similarly there was significant increase in the activity of catalase in blood and liver. Decrease in glutathione peroxidase biolox and administration was found to be increased significantly in the blood and liver from the 9th day by extract treatment. Glutathione reductase was also found to be increased in blood and liver. These results suggest that terminalia bellerica fruit extract pocessed Anti Diabetic and antioxidant activity and these activities may be interrelated.

Antidiabetic and antioxidant activity of terminalia bellerica: Roxb

Sabu MC,et.al.Indian exp Biol.2009

**Author information:**

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**Citation:**

Indian JExp Biol.2009 Apr; 47(4) 270_5.

# 3.5. DISEASE REVIEW

## 3.5.1. Madhu Megam - Siddha Aspect

**Synonyms:**

*Madhu premegam*

*Inippu Neer*

*Neerizhivu*

*Meha neer*

**Definition**:

*Madhumegam* is a clinical condition characterized by frequent urination resulting in deterioration and diminution of seven thathus and loss of weight, enormous ants and flies on the place where urine is expelled and on heating it gives the smell of sugar.

Abdomen distends like sea, slurring of speech, peripheral neuritis, lassitude, dyspnoea are the symptoms of *madhumegam*.

**Etiology:**

**1.Dietary causes:**

"கோதையர் கலவி போதைகொழுத்தமீ னிறைச்சிபோதை
பாதுவாய் நெய்யும் பாலும் பரிவுடனுண்பீ ராகில்
சோதபாண் டுருவ மிக்க சுக்கில பிரமேகந்தான்
ஓதுநீ ரிழிவுசேர வுண்டென அறிந்து கொள்ளே

***- Agasthiyar – 1200***

Excessive intake of fatty fish, meat, alcoholic, toddy , etc., causes diabetes Milk, ghee, sweet foods, uncooked food, chilled rice when taken in large quantity leads to this disease.

**2. Sexual indulgence:**

1. ஸ்திரி போகம் செய்ததினால் வேவு கொண்டு
சிரசு மட்டும் வெந்துடுகிக் கனலே மீறிக்
குறியுடனே மேகந்தான் கொடுமை செய்து
குறைந்து வரும் தாதுவெல்லாம் குன்றிப் போகும்

***- Guru Nadi***

கன்னி மயக்கத்தால் கண்டிடு மேகமே நாடி நூல்
…… …… மங்கை கோட

***- Yugi Vaithiya Chindhamani***

"நிறை பூத்து கொங்கையான் நாயகன் மோகத்தால்
மறை போற்றும் கருப்பத்தில் வளாத்து மேகமே"
"கிரந்தி புண்ணிரன மேகக் கீசகனெனுந் துன்மார்க்கன்
அருந்ததி என்னும் பாஞ்சாலி யன்னையைக் கண்ணுற்றானே"

***-Thaeran arundhu Bharadham***

Excessive sexual indulgence may leads to diabetes.

**Humoral changes:**

"பகர்பித்த விந்தையலாது மேகம் வராது"

Unless pitham rises no one is affected by *megam.*

"இனிக்கின்ற வாதத்திடை சேரில் ஐயந்தான்
மனிக்கின்ற கள்ளுப் பதனிபோல் நீரோடும்
கனிக்கின்ற மேனி கரைந்து வெளுப்போதும்
தனிக்கும் மதுமேகந் தப்பாதே பையமே"

***- Thirumoolar Vaithiyam – 600***

If vatham is accompanied by kapham urine excreted resembles *kallu* or *padhini*. Body gets emaciated and becomes pale and Madhu megam will develop without fail.

***Karma noi:***

"ஆமப்பா மனிதர் செய்த காம்த்தாலே
அரகரா மேக மென்ற ராசாவாலே
காமப்பா லதினால் பசியுப்ப நாலுங்
கைக்கடங்கல நோய்கள் வரும் காம்த்தாலே"

***-Agasthiyar***

Deeds of a man are responsible for the development of Megam, which presents with increased hunger and by the disease gets out of control.

**Psychosomatic Factor:**

"மதங்கொண்டு பெரியோரை வைகையாலும்
மாதர் கற்புநிலைமை தன்னை அழிக்கையாலும்
பதங்கொண்ட சிவயோகி சாபத்தாலும்
பத்துவகை சிலேற்பனங்கள் மேகநீராம்"

***-Yugi Muni***

Getting mad and scolding elderly people, abusing female, curse of a devotee of Lord Shiva causes 10 types of *selaerpana mega neer.*

**Signs andSymptoms:**

"மிக்கான சடம் வெளுத்து மேனி கண்ணும்"

***-Yugi Muni***

Body becomes pale and emaciated.

"குறியுடனே மேகந்தான் கொடுமை செய்து
குறைந்து வரும் தாது வெல்லாம் குன்றி போகும்"

***- Guru Nadi***

The body humors decreases, hence it gets emaciated

**CLASSIFICATION OF DISEASE:**

*Yugi vaithiya chindamani*-20 types

***Vadham-4***

*Achiyakandhi megam*
*Sutha megam*
*Vatha pramiya megam*
*Mamisa siravi megam*

***Pittham-6***

*Appiya megam*
*Pitha pramiya megam*
*Savirana megam*
*Madhu megam*
*Chandira megam*
*Arakka megam*

***Kapha megam: 10***

*Vasa megam*
*Uthama megam*
*Maghai megam*
*Adhikka megam*
*Sura megam*
*Sukkila megam*
*Udhaya megam*
*Presa megam*

*Sara megam*

*Thaithiya megam*

***Madhu Mega Avathaigal* (Complications of Diabetes)**:

காணவே முதலவத்தை சரிரந் நானும் கணமாகப் பரத்திற்கு நீர்த்து வாரம்
வேணவே வெண்டாக்கி யகலம் பண்ணும் மிக்கவிரண் பாமவத்தை விளம்பக் கேளாய்
மூணவே முத்ரபத் டையுமாச் சுக்ல முகமழுகி தேஜசுதான் மிகவே குன்னும்
நாணவே மூன்றாகு மவத்தைக் குந்தான் தாவாளும் வாவுவது மீறுந் தானே
"தானான நாலவத்தை யங்க தாகம் சன்னியது பாதமுண்டாமைந்த வத்தை
தேனான நீர்பெருகுந் தாது நட்டம் நிலையாறா மவுத்தையுடற் கிடைகொள்ளாது
மூனான மூர்ச்சை வருமேள வத்தை மிக்கவரோ சிகன்ஞ்சுவசந் தேகசாட்யம்
ஞனான எட்டாவ தவத்தை தானே எழுகிரந்தி பிளவையுந்தான் மிகவுண்டாமே.
உண்டாகு மொன்பதா மவத்தை கேளாய: ஒழுக்கான ஆசாரங் கிருமி யுண்டாம்
மண்டான பத்தாந்தா னவத்தை கேளாய் பாரமாம் கூடியங்கண்டு பரத்துக் கேடும்
வெண்டாகு மேகந்தா னிருப துக்கும் விளங்கியதோர் தச அவத்தை விபரஞ் சொன்னோம்"

***-Yugi Vaithiya Chindhamani***

1st Avathai - Body becomes obese and urethra gets dilated.

2nd Avathai - Increased urination and quality of sperm is affected.

3rd Avathai - Dryness of tongue, fullness of stomach.

4th Avathai - Increased thirst and delirium develops.

5th Avathai - Increased micturition and destruction of sperm.

6th Avathai - Difficulty in lying down and dyspnoea

7th Avathai - Tumors develop in the body.

8th Avathai - Nausea, loss of appetite, hyper apnea and lethargy

9th Avathai - Microorganisms develop in the body and it becomes thin

10th Avathai - TB develops.

## 3.1 MODERN ASPECT

**Diabetes Mellitus (*Madhu Megam*)**

**Definition:**

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia with or without glycosuria, resulting from an absolute or relative deficiency of insulin. This is brought about by an impairment of insulin production or its release by the beta cells of the islets of Langerhans. [Diabetes (Greek)-to pass through, Mellitus (Latin)-honeyed or sweet.]

**Types:**

1. **Primary Diabetes**:

   Type 1 (IDDM)

   Type II (NIDDM)

2. **Secondary Diabetes:**

   Etiology:

   Pancreatic Diseases (pancreatitis, cystic fibrosis, neoplastic diseases, pancreatomy, haemochromatosis, fibrocalculus pancreatopathy)

   Viral Infections (Congenital rubella, Mumps, Coxsackie-B virus)

   Drug Induced(Corticosteroids, Phenytoin, Thiazide diuretics)

   Associated with genetic syndrome (Down's syndrome, Turne's syndrome, Wolfram's syndrome).

   Production of hormonal antagonists to insulin (Growth hormone-acromegaly, Glucogon-glucogonoma,

   Glucocorticoids-cushing's syndrome, Thyroid hormone-thyrotoxicosis, Catecholamines- phaeochromocytoma)

**3.Gestational Diabetes:**

About 4% pregnant women develop DM due to metabolic changes during pregnancy. Although they revert back to normal glycemia after delivery, these women are prone to develop DM later in their life.

**Normal Insulin Metabolism**:

The major stimulus for both synthesis and release of insulin is glucose. The steps involved in bio synthesis, release and actions of insulin are as follows

**Synthesis:**

Insulin is synthesized in β cells of pancreatic islets of Langerhans

It is initially formed as pre pro insulin.

Subsequent proteolysis removes the amino terminal signal peptide, forming pro insulin.

Further cleavage of proinsulin give rise to A (21 amino acids) and B (30 amino acids) Chains of insulin, linked together by connecting segment called C peptide.

**Release:**

Glucose is the key regulator of insulin secretion from β cells by a series of steps:

Hypoglycemia (glucose level below 70 mg/dl) stimulates transport into β cells of a glucose transporter, GLUT2.

An islet transcription factor, glucokinase causes glucose phosphorylation.

Metabolism of glucose to glucose 6- phosphate by glycolysis generates ATP.

Generation of ATP alters the ion channel activity on the membrane which stimulates insulin release.

**Action:**

Half of insulin is secreted from βcells into portal vein is degraded in the liver while the remaining half enters the systemic circulation for action on the target cells.

-Harsh mohan text book of pathology

**Prevalence:**

| | | |
|---|---|---|
| Type 1 | - | 5-10% |
| Type 2 | - | 90-95% |
| Gestational diabetes | - | 2-5% |
| Other specific types | - | 2% |

On digestion, carbohydrates are converted into glucose and absorbed into the blood. When intestinal glucose absorption declines between meals; hepatic glucose output is increased. The glucose in the blood is essential for all the cells as it acts like a fuel. The glucose uptake occurs by two different mechanisms.

Insulin dependent-most of the organs require insulin for glucose uptake

Insulin independent- some tissue like nerve, lens, kidney, blood vessels, beta cells of pancreas.

As stated above β-cells of pancreas do not require insulin for glucose uptake. Hence when blood sugar rises, it enters the β-cells of pancreas and stimulates the release of a substance called pre-pro insulin by the process of translation of messages

present in m-RNA which occurs in rough-endoplasmic reticulum. It is proteolytically cleaved in the golgi apparatus, to form insulin and C-Peptides. Both of them are stored in secretory granules. Again insulin acts as a trigger for insulin release.

**Type 1 Diabetes (IDDM)**

This form of diabetes results from a severe, absolute lack of insulin caused by reduction in the beta –cells mass. IDDM usually develops in childhood, becoming severe at puberty. Hence it was previously known as Juvenile- onset diabetes mellitus. The patient depends on insulin for survival. Without insulin, they develop acute metabolic complications, such as ketoacidosis and coma. Hence it is usually called ketosis –prone diabetes.

**Pathogenesis:**

Three interlocking mechanisms are responsible for islet cell destruction.

**1.Genetic Susceptibility**:

Genetic factors account for one-third of the susceptibility to type I diabetes. The short arm of chromosome 6 contain a human leucocyte antigen (HLA) region.The HLA class 2 genes code for proteins on surface of cells, which present normal β-cell antigens to T lymphocytes. Defective presentation of these auto antigens underlies susceptibility to autoimmune disease.

**2.Autoimmunity:**

Approximately 10% of persons who have type 1 DM also have other organ-specific autoimmune disorders, such as Grave's disease, Addison's disease, Thyroiditis and Pernecious anaemia. In these patients, there appears to be a broad of immune regulation.

**Environmental Factors:**

Reduced exposure to microorganisms in early childhood, limits maturation of immune system and increased susceptibility to autoimmune diseases. Viruses cause mild β cell injury, which is followed by autoimmune reaction against altered β cells in persons with HLA susceptibility. Cow's milk contain Bovine Serum Albumin (BSA), which when fed to children cross the neonatal gut and raise antibodies , which not only destroys BSA but also β cells, because of close homology or resemblance between the two (i.e) destruction by the process of molecular mimicry. Nitrosamines found in smoked meat and coffee has been proposed as potential diabetogenic toxin. Stress may precipitate type 1, by stimulating secretion of counter-regulatory hormones and also modulating immune activity.

**Type 2 Diabetes:**

It was previously called as adult-onset diabetes. Initially insulin resistance appears and leads to elevated insulin secretion in order to maintain normal blood glucose level. However, in susceptible individuals the pancreatic β cells are unable to sustain the increased demand for insulin and a slowly progressive insulin deficiency develops.

**Pathogenesis:**

The underlying causes are largely unidentified genetic factor and the effects of a western lifestyle such as obesity and overeating.

**1.Insulin Resistance:**

The primary cause of insulin resistance remains unclear but presence of obesity is a powerful amplifier of insulin resistance. Decrease in number of insulin receptors, postreceptor defect, reduced synthesis or translocation of GLUT-4 in muscles and fat such as Free Fatty Acids (FFA's), found in large quantities in obese, competes with glucose as a fuel supply for oxidation in the periphery. Certain hormones from visceral adipose tissue drain into the portal vein and affects insulin sensitivity in liver. Inactivity is associated with down regulation of insulin-sensitive kinases and also increased accumulation of FFA's within skeletal muscles. Sedentary people are therefore more insulin –resistant.

**2. Pancreatic β cell Failure:**

Amylin, a polypeptide is secreted together with insulin. So that, in presence of insulin resistance, excessive demand for insulin secretion also results in formation of excess amylin which forms insoluble fibrils of amyloid and ultimately destroy β cells. Reduction of GLUT-2 transporters, which facilitate glucose entry into β cells, is caused by chronic hyperglycemia, referred to as glucose toxicity.

**3.Genetic predisposition:**

Genetic factors are important in the etiology of type2 diabetes. Unlike type 1, however, the disease is not linked to any HLA haplotype and there is no evidence that autoimmune mechanisms are involved. Regions that contain susceptible loci on chromosome 1q,12q and 20q have been identified. But the underlying gene have not been identified. In MODY, there is autosomal dominant inheritance of a single gene linked to chromosome 7 and 20

**4.Environmental Factors:**

Overeating, especially when combined with obesity and under activity causes type2 DM. Sweet foods rich in refined carbohydrate ,consumed frequently may increase the demand for insulin secretion, while high-fat food may increase FFA's and exacerbate insulin resistance.

**Gestational Diabetes:**

Glucose metabolism changes during normal pregnancy, reflecting altered glucose handling by the mother and nutritional demands of developing fetus. In normal women, marked insulin resistance develops, particularly by the second half of the placental hormone activity. Fasting glucose decreases slightly while blood glucose may be increased post-prandially.

Gestational diabetes is defined as diabetes with first onset or recognition during pregnancy. While this includes women with preexisting type 1 and type 2diabetes. The majority can expect to be restored to normal glucose tolerance immediately after pregnancy. Repeated pregnancy may increase the likely hood of developing irreversible diabetes, particularly in obese women. 80% of women with gestational diabetes ultimately develop permanent diabetes.

**Main Clinical Features:**

1. Polyuria
2. Polydipsia
3. Polyphagia
4. Fatigue

**Other Clinical Features:**

**1. Unexplained weight loss:**

Though they eat more, they are unable to process many of the calories in the food. Factors contribute to weight loss:

Losing sugar and water in urine and accompanying dehydration

Lipolysis which deplete fat stores.

**2.Poor wound healing:**

Impaired WBC function (due to increased glucose levels)

Decreased peripheral blood flow.

Glucose laden tissue which offers a media to grow.

3. Very dry skin due to dehydration.

4. Sudden vision changes.

5. Tingling or numbness in hands or feet.

6. Recurrent infections due to decreased immunity.

7. Nausea, vomiting and stomach pain may develop abrupt onset of type I diabetes.

8. Altered mental status

Other Agitation, unexplained irritability, lethargy and confusion may develop due to that the brain cells cannot oxidize free fatty acids and is easily affected by increased blood sugar, hyper osmolarity and hypoglycemia

- K.V.Krishna Das text book of medicine

**Table No: 3 Diagnostic criteria of pre diabetes**

| **Criteria for Diagnosis of Pre Diabetes** | | |
|---|---|---|
| **Category** | **Fasting plasma glucose** | **Postprandial (2 hours)** |
| 1.Normal<br>2.Impaired fasting glucose (IFG)<br>3. Impaired glucose tolerance(IGT) | <100 mg/dl<br>100-125mg/dl<br><br><100 mg/dl | < 140 mg/dl<br>< 140mg/dl<br><br>140-200mg/dl |

**Table No: 4 Clinical Features of Type 1 and Type 2 DM**

| **Clinical features** | **Type 1** | **Type 2** |
|---|---|---|
| Usual age at onset | 5-30 years | 35-65 years |
| Sex | M:F 1:1 | F>M in India |
| Prevalence Among diabetes | 15-18% in Caucasians 1% in Indians | 95-98% of Indians |
| Genetic | Hereditary+StrongHLA association | Hereditary +No HLA association |
| Nutrition | Lean | Often obese |
| Usual onset | Rapid with classic symptoms | Slow, insidious, often with only mild symptoms |
| Ketosis | Prone | Not prone |
| Islet cell antibodies | Maybe present within 1yr of onset | Absent |
| Treatment modality | Almost all require insulin for control of sugar | Majority can be controlled by oral hypoglycemic drugs |

**Complications:**

**1.Diabetic Microangiopathy**:

It is most marked in type 1, developing in early life but also occurs in type2. Various factors like endothelial damage, increased plasma viscosity, erythrocyte aggregation, reduced red cell deformability and increased platelet adhesion lead to micro-angiopathy. It affects several organ systems. The main lesions are seen in the retina, kidneys, peripheral nerves and heart giving raise to diabetic retinopathy, nephropathy, many forms of diabetic neuropathy and cardiomyopathy.

**2. Atherosclerosis:**

HDL levels are reduced in type 2, possibly enhancing susceptibility to atherogenesis. AGE formation also contributed to atherosclerosis. Diabetics have increased platelet adhesiveness and most of type2 patients are obese.

**3. Diabetic Nephropathy:**

It is common complication and and a leading cause of death in DM.

Diabetic glomerulosclerosis

Vascular lesions

Diabetic pyelonephritis and necrotizing renal papillitis

Tubular lesions or Armanni-Ebstein lesion

**4. Diabetic Neuropathy:**

Disordered glucose metabolism and micro angiopathy affecting nutritional maintanence of peripheral nerves are responsible for neuropathies. Glove and stocking impairment of all other modalities of sensation developes. Toes may be clawed. Callus skin develops at metatarsal heads due to increased pressure.

**5. Diabetic Retinopathies:**

DM produces a classical retinopathy. A specific change occurs in the vessels leading to loss of mural cells (pericytes) and the formation of micro aneurysms. Once initiated the fundus changes are usually progressive. The early changes are venous dilation and the appearance of small dot like micro aneurysms in the perimacular area. Atrial blood is shunted and this leads to ischemia of the retina.

Large subhyaloid hemorrhages and vitreous hemorrhages may develop and the vision is seriously impaired. Such hemorrhages are due to rupture of newly formed blood vessels. As these hemorrhages are absorbed, organization by fibrous tissue results and multiple bands of retinitis proliferation develop. These lead to permanent visual impairment. Retinopathy is usually associated with advanced neuropathy.

Some times in DKA with severe hyperlipidemia the fat gives a milky white appearance to the retinal arteries called “lipemia retinalis.”

**6. Diabetic foot:**

Foot problems give raise to severe morbidity in diabetic patients. DM accounts for up to 50-80% for non-traumatic amputations. The factors contribution to the foot problems are:

Neuropathy - sensory, motor and autonomic.

Ischaemia

Trauma – physical, mechanical, thermal, continuous mechanical stress, uneven weight bearing.

Infection

Ulceration of the foot in a diabetic usually results from the combination of neuropathic damage, tissue ischaemia and excessive pressure loading, often with supreme post infection.

**7. Complications in Pregnancy and Neonates:**

There may be miscarriages and abortions, toxaemias of pregnancy, hydraminosis, etc. Herculian (macrosomia) child may be born of diabetic mother due to excess of growth hormone and maternal hyperglycaemia.

**8. Diabetic ketoacidosis:**

The three cardinal biochemical features of DKA – hyperglycaemia, ketosis and acidosis results from the combined effects of deficient circulating insulin activity and the excessive secretion of counter regulatory hormones. The end results are hyperglycemia (>250 mg/dl), ketoacidosis (pH<7.30), and an osmotic diuresis that promotes dehydration and electrolyte loss.

**9. Non-ketotic hyperglycemic hyperosmolar coma:**

It is a serious emergency seen occasionally in the elderly diabetes. It is commonly precipitated by infections, MI, burns, trauma, surgical stress, renal failure, pancreatitis and the use of certain medications.

**10. Lactic acidosis**

Normal level of serum lactate is between 0.4 and 1 mmol/L in the fasting state and it may go upto 2mmol/L, after meals 5-18mg/dl this is maintained by the balance between production and utilization of lactate. In diabetic lactic acidosis the blood lactate level is increased above 5mmol/L

The hypoxic state produced by aberration in tissue metabolism probably contributes to the lactic acidosis associated with severe diabetes.

**Side effects of insulin therapy:**

Hypoglycemia

Weight gain

Peripheral oedema insulin treatment causes salt and water retention in the short term

Insulin antibodies local allergy rare

Lipodystrophy at injection sites.

- davidsons principle and practice of medicine

# 3.6. PHARMACEUTICAL REVIEW

## 3.6.1. SIDDHA ASPECT

### PARPAM (CALX)

**Definition:**

*Parpam* is equivalent to calx, which is prepared by the process of calcination. The correct Tamil translation would be "*Neeru*" which would mean an ash. "*Saambal*" is another word equivalent to an ash or calx.

Metals, *uparasas* or *paashanas* are made into white powder by the process of *pudam*, burning, frying, blowing and by grinding them with juices, *ceyaneer* etc.

**Equipment required:**

Mortar and pestle.

Vessels and spoons to handle liquids.

Long ribbons of tough cloth and fine clay.

Pairs of shallow earthen discs of identical dimensions.

Cow dung cakes, sufficient numbers and well dried.

Fine cloth pieces for filtering juices and decoction.

Spatulae for handling powders.

**General method of preparation:**

The drugs are ground according to the particular recipe, with other drugs, juices or decoctions, and the resultant mass is made into small, thin circular cakes and dried .When they are well dried, they are taken for calcination.

The material ready for calcination, is put into an earthen disc described earlier, and covered by inverting another disc and sealing the rim with the cloth ribbon one side of which is smeared with wet clay. This makes a capsule type crucible. When the seal is dry, the capsule is placed in the kiln for calcination.

The kiln or "*pudam*" as it is called in Tamil, is made by digging a pit of appropriate dimensions in the soil, and filling it with the recommended number of cowdung cakes, which is the fuel. It is better that the interior of the pit is lined with bricks, so that the pit could be used repeatedly.

Seventy five percent of the recommended numbers of dung cakes are arranged in the pit and then the capsule is placed in the centre. The rest of the dung cakes are arranged above this and the top is somewhat dome shaped. When some burning

charcoal pieces are placed on the dome, the dung cakes below them catch fire and the fire spreads all around in a uniform manner.

The kiln will burn for a long time, until all the dung cakes are burnt and converted into ashes. When the kiln cools down, the ashes are very carefully removed and the capsule is taken out without damaging the seat. The exterior of the capsule is thoroughly brushed to remove the ashes and the seal is scraped off and removed.

The contents of the capsule are recovered, and the remnants that adhere to the walls are gathered by great scraping and brushing.

For the complete transformation of the material into "*parpam*" state, the process of grinding, drying and calcination may have to be repeated several times or atleast as many times as directed in the recipe. However, the calcination is repeated until a satisfactory product is obtained. But in those instances where the number of calcinations is definitely indicated, the process should be repeated accordingly, even if a satisfactory "*parpam*" is obtained within a few calcifications.

**Colour:**

In general *parpam* are always white in colour but *Tankaparpam* (calx of Gold) is light yellow in colour.

**Character and tests for *parpam*:**

The final product should not have any glitter or shine.

If a small quantity is pinched and rubbed between the thumb and index finger, the particles should be so fine as to enter and reside in the furrows and folds.

If a pinch of *parpam* is gently put on the surface of water kept in a container, the material should not sink, but it should float.

If the *parpam* is put into a crucible and heated, it should not be reverted into its metallic state.

**Preservation and storage:**

*Parpam* should be stored in a clean dry and air tight glass containers.

**Shelf life:**

*Parpam* keep their potency for 100 years.

**Note:**

The colour effect and fineness of the *parpam* will be enhanced according to the degree of grinding and so it should be ground very finely.

If the discs are not well dried, the *parpam* will not attain the specific colour specific for the particular *parpam*.

When the discs (Dried *villai*) are arranged in the pans they should not be heaped up and should not be arranged in more than one layer. Only then the heat will react on the material properly. The pans should not be disproportionately big when compared to the quantity of the drugs and they should not be very deep also.

The kiln is constructed by making circular excavations of suitable dimensions in places with optimum aeration and the sides are lined with bricks. Kilns should not be constructed in places where strong winds blow.

Half of the numbers of cow dung cakes are spread at the bottom of the kiln and the calcination earthen vessels are placed over this at the centre. The remaining cow dung cakes are arranged over this and are to be ignited all around.

Usually cow dung cakes are used as fuel in kilns. However in some specified instances some barks or goat dung and other materials are also recommended.

In cow dung cakes there will be an appreciable admixture of sand or mud.Depending upon the degree to which there is such admixture the number and weight of the cow dung cakes are increased.One can determine this correctly only by adequate practical experience.

Materials like sulphur and yellow orpiment which do not withstand strong heating are hidden in specified ashed when being calcined. In such cases the ash is spread in the pan, the discs placed over and then covered with more ash, after which the other pan is inverted over and scaled along the seam.

The calcination capsules and the contents there in should be taken only when the kiln has cooled down by itself.

# 4. MATERIALS AND METHODS

## 4.1 PREPARATION OF THE TRIAL DRUG

**Selection Of Drug:**

The Drug *"komoothira silasathu parpam"* has been selected for Hypoglycemic, Anti Dyslipidemic and Anti oxidant activitities from the Classical Siddha literature*"Thanjai vaithiya Raja Sinthamani* authored by Rajasree Sathrapathi Saraboji maharaj Saheb. Page no: 5.

**Ingredients Of The Drug:**

*1. Komoothira silasathu(*Asphaltum*)*

2.*Kadukkai(Terminalia chebula)*

*3. Thandri thol(Terminalai bellarica)*

*4. Nelli mulli(Phyllanthus emblica)*

**Collection Of The Drugs**:

The *Komoothira silasathu* (Asphaltum) was bought from the raw drug shop at Ramaswamy chetty traders, Chennai, Tamilnadu.

The *Kadukkai (Terminalia chebula)*, *Thandri thol (Terminalai bellarica), Nelli mulli (Phyllanthus emblica)*were also bought from Ramaswamy chetty traders, Chennai, Tamilnadu.

**Identification And Authentication:**

All raw drugs were identified and authenticated by the experts of *Gunapadam* (pharmacology) and Medicinal botany department in Government Siddha Medical College Palayamkottai, Tirunelveli.

The specimen samples of the identified raw drugs were presented in the laboratory of PG Gunapadam for future references.

**PURIFICATION OF RAW DRUGS:**

***Komoothira silasathu*(*Asphaltum*):**

First *komoothira silasathu* was taken, powdered then dissolved in hot water, filtered in a white cloth and is kept in a glass bowl. Then this is exposed to sunlight. The outer layer is collected and stored.

*Kadukkai:*

Remove the seed from *Kadukkai* and dried.

*Thandri thol:*

Remove the seed from *thandrikai* and dried.

*Nelli MullI:*

Remove the seed from *Nellikai* and dried.

**Method Of Preparation:**

**Process of preparation :**

Dried pericarp of Terminalia chebula, Terminalia bellarica, Emblica officinalis each weighing about 42 gm taken in a clean pot and add 1600ml of water. It was boiled and reduced upto 200ml and filtered. And then 84 gms of purified *komoothira silasathu* is placed in a stone mortar and triturated with above prepared decoction for about 3 hours and made onto small cakes and dried in sunlight.

Then dried cakes (villai) were placed with in a earthern plate and covered with another earthen plate and subjected to incineration process by using dried cow dung cakes. After incineration process it is allowed to cool. The cakes are taken and grind into fine powder. The above incineration process is repeated for 5 times by grinding with aove said decoction.Finally the white coloured *Komoothira Silasathu parpam* is obtained and stored in a container

**Shelf Life**:

100 years.

**Dosage**:

*Panavedai* (488mg) , 2times

**Adjuvent:**

*Nerinjil mul kasayam*

**Indication:**

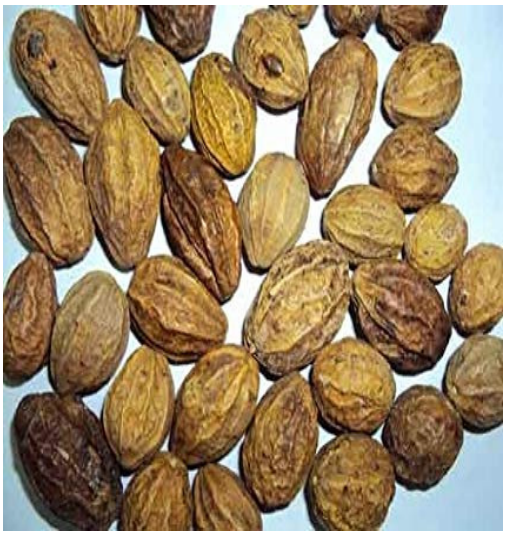
*Madhumegam ,megam 21, muthirakiricharam, Jeeranasuram, Hashmarirogam.*

**Fig No: 1**

**INGREDIENTS OF *Komoothira Silasathu Parpam***

***Komoothira Silasathu***

***Kadukkai***



***Nellimulli***

***Thandrikkai***

PURIFICATION PROCESS

**Fig No: 2**

**FINAL PRODUCT**

***Komoothira Silasathu Parpam***

# 4.2.STANDARDIZATION OF THE DRUG

## 4.2.1 .AS PER *SIDDHA* CLASSICAL LITERATURE:

Standardization of drug means confirmation of it's quality and purity and detection of the nature of adulterant of various parameters like morphological, microscopic, physical, chemical and biological observations.

**1. Colour:**

The finished form of *parpam* is generally white in colour.

**2. Odour:**

Wherever a specific odour has been observed it has been mentioned, where a characteristic odour is said to be present it is examined by smelling the drug directly after opening the container.if such an odour is discernible, the contents are rapidly transferred to an open and re examined after 15 minutes. If odour persists to be discernible, the sample complies with the description for odour

**3. Taste:**

The taste of a drug is examined by taking a small quantity of drug by the tip of moist glass rod and allowing it on tongue previously moistened with water. This does not apply in the case of poisonous drugs.

**4. Finger Print Test:**

Well prepared *parpam* should be very fine. A pinch of *parpam* was taken and rubbed in between the thumb and index finger. It entered into the depressions and furrows of the fingers.It confirmes the fineness of *parpam.*

**5. Floating on Water**:

A pinch of *parpam* was sprinkled over the water in a glass container. The *parpam* particles did not sink but floated on the water surface. It indicates the liteness of *parpam*.

**6. Lustre:**

If any glowing particles seen in the *parpam* , it shows that the drug is not prepared properly and possess unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *parpam*. The *parpam* was taken in a Petri dish and observed for any lustre in daylight via magnifying glass.

## 4.2.2. STANDARDIZATION OF TEST DRUG BY USING MODERN TECHNIQUES:

Standardization of drug helps to authenticate and determine its quality and efficiency. Thus, the process involves qualitative and quantitative analysis by means of physico – chemical properties such solubility test, Ash test, Flame test etc and instrumental analysis.

The physico – chemical analysis of *Komoothira silasathu parpam* has been done in IIT Lab- chennai

The chemical finger prints are engaged by using modern analytical technique Fourier Transform Infra –Red Spectroscopy (FTIR) and Powder X-ray diffraction methods.

The particle size and qualitative analysis of chemical elements of *Komoothira silasathu parpam* are also assessed by Scanning Electron Microscope (SEM) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

## 4.2.2.1. PHYSICO – CHEMICAL ANALYSIS:

**Solubility test:**

A. A little amount of the sample was taken in a dry test tube and shaken well with distilled water.

B. A little amount of the sample was taken in a clean dry test tube and then shaken well with con HCl and Con.H2SO4. Sparingly soluble character of the sample indicates the presence of Silicate.

**Action on heat:**

A small amount of the sample was taken in a clean dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

**Flame test:**

A small amount of the sample was taken in a clean dry watch glass. It was made into paste with concentrated HCL. And then it was introduced into non-luminous part of the Bunsen flame. If bluish green flame appears, it indicates the presence of copper.

**Ash test:**

A small amount of sample was mixed with the cobalt nitrate solution. A filter paper was soaked into the mixture. Then it was introduced into the Bunsen flame and ignited. If yellow colour flame appears, it reveals the presence of sodium.

**Determination of Total Ash:**

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450º until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug.

**Determination of Acid Insoluble Ash:**

Boil the ash obtained in above test for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

**Determination of Moisture Content (Loss on Drying):**

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowdered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

## 4.2.2.2 MICROBIOLOGICAL CONTAMINATION

**Evaluation of Total Aerobic Bacterial Count**

**1.1.Preparation of Sample for Experimental Work**

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

**1.2. Serial dilution of Sample**

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as $10^{-1}$ dilution. From this dilution, one ml was taken from that mixture is added to 9 ml, and designated as $10^{-2}$ dilution. The same procedure was repeated up to $10^{-4}$.

**1.3. Isolation of Total Viable Aerobic Microbial Count**

**1.3.1. Isolation of Bacteria by Plate Count Method**

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45$^{o}$C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of $10^{-1}$ and $10^{-2}$. After inoculations, all the plates were incubated at 37$^{o}$C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected.

**1.3.1.1. Composition of Nutrient Agar Media**

| | |
|---|---|
| Peptone | : 5.0 gm |
| Sodium chloride | : 5.0 gm |
| Beef extract | : 1.5 gm |
| Yeast extract | : 1.5 gm |

| | |
|---|---|
| Agar | : 15.0 gm |
| Distilled water | : 1000 ml |
| pH ( at 25°C) | : 7.4±0.2 |

**1.3.2. Isolation of Fungi**

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

**1.3.2.1. Composition of SDA**

| | |
|---|---|
| Dextrose | ; 40 gm |
| Peptone | : 10 gm |
| Agar | : 15 gm |
| Distilled water | : 1000 ml |

**1.5. Evaluation of Specified Microorganisms**

**1.5.1. Isolation & Identification of *Escherichia coli***

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

**1.5.1.2. Primary Test**

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

**1.5.1.3. Secondary Test**

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

**1.5.1.4. Alternative test**

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the pink or brick red color colonies were examined and transfer them individually into the surface of Eosin Methylene Blue

agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

**1.5.1.5. Components of Eosin Methylene Blue Agar Media**

| | |
|---|---|
| Pancreatic digest of gelatin | : 10.0 g |
| Dibasic potassium phosphate | : 2.0 g |
| Lactose | : 10.0 g |
| Eosin Y | : 400 mg |
| Methylene blue | : 65 mg |
| Agar | : 15.0 g |
| Distilled water | : 1000 ml |

**1.5.2. Isolation & Identification of *Salmonella* sp.**

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

**1.5.2.1. Primary Test**

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

**1.5.2.2. Secondary test**

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

**1.5.2.3. Composition of *Salmonella Shigella* Agar Media**

| | |
|---|---|
| Beef Extract | : 5.0 gm |
| Enzymatic Digest of Casein | : 2.5 g |
| Enzymatic Digest of Animal Tissue | : 2.5 gm |
| Lactose | : 10 gm |
| Bile salts | : 8.5 gm |
| Sodium Citrate | : 8.5 gm |
| Ferric Citrate | : 1.0 gm |
| Brilliant Green | : 0.00033 gm |
| Neutral Red | : 0.025 |
| Agar | : 13.5 gm |
| Distilled water | : 1000 ml |

**1.5.3. Isolation and Identification of *Pseudomonas aeruginosa***

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

**1.5.3.1. Composition of Cetrimide Agar Media**

| | |
|---|---|
| Pancreatic digest of gelatin | : 20.0 g |
| Magnesium chloride | : 1.4 g |
| Potassium sulphate | : 10.0 g |
| Cetrimide | : 0.3 g |
| Agar | : 13.6 g |
| Glycerin | : 10.0 g |
| Distilled Water t | : 1000 ml |

**1.5.4. Isolation and Identification of *Staphylococcus aureus***

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

**1.5.4.1. Composition of Mannitol Salt Agar Media**

| | |
|---|---|
| Pancreatic digest of gelatin | : 5.0 g |
| Peptic digest of animal tissue | : 5.0 g |
| Beef extract | : 1.0 g |
| D-Mannitol | : 10.0 g |
| Sodium chloride | : 75.0 g |
| Agar | : 15.0 g |
| Phenol red | : 25 mg |
| Distilled Water | : 1000 ml |

## 4.2.2.3. BIO CHEMICAL ANALYSIS

**PROCEDURE:**

100mg of the drug was wighted accurately and placed into a clean beaker added a few drops of cons. hydrochloric acid and evaporated it well. After evaporated cooled the content and added a few drops of concentrated nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transformed it to 100 ml of volumetric flask and made upto100 ml with distilled water. Mix well. Filtered it.Then it taken for analysis.

**QUALITATIVE ANALYSIS FOR BASIC RADICALS:**

**Test for Calcium:**

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

**Test for Iron (Ferric):**

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

**Test for Iron (Ferrous):**

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

**Test for Zinc:**

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the absence of zinc.

**QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:**

**Test for Sulphate:**

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

**Test for Chloride:**

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

**Test for Phosphate:**

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of no yellow precipitate indicates the absence of phosphate.

**Test for Carbonate:**

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

**Test for starch:**

The extract is added with weak iodine solution. Formation of no blue colour indicates the absence of starch.

**Test for albumin:**

The extract is treated with Esbach`s reagent. Formation of no yellow precipitate indicates the absence of albumin.

**Test for tannic acid:**

The extract is treated with ferric chloride. Formation of nobluish black precipitate indicates the absence of tannic acid.

**Test for unsaturation:**

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

**Test for the reducing sugar:**

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

**Test for amino acid:**

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

## 4.2.2.4.INSTRUMENTAL ANALYSIS

**Scanning electron microscope (SEM)**

Fig No: 3 scanning electron microscope (sem)

**Introduction:**

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo.

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter.

**Principle:**

The beam is then rastered over the specimen in synchronism with the beam of a cathode ray tube display screen. The eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

**SEM MECHANISM**

**Procedure:**

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing secondary electronsalso increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases

beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

**Applications:**

Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *Komoothira silasathu parpam*. SEM results of *Komoothira silasathu parpam* were represented in results section.

# FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)

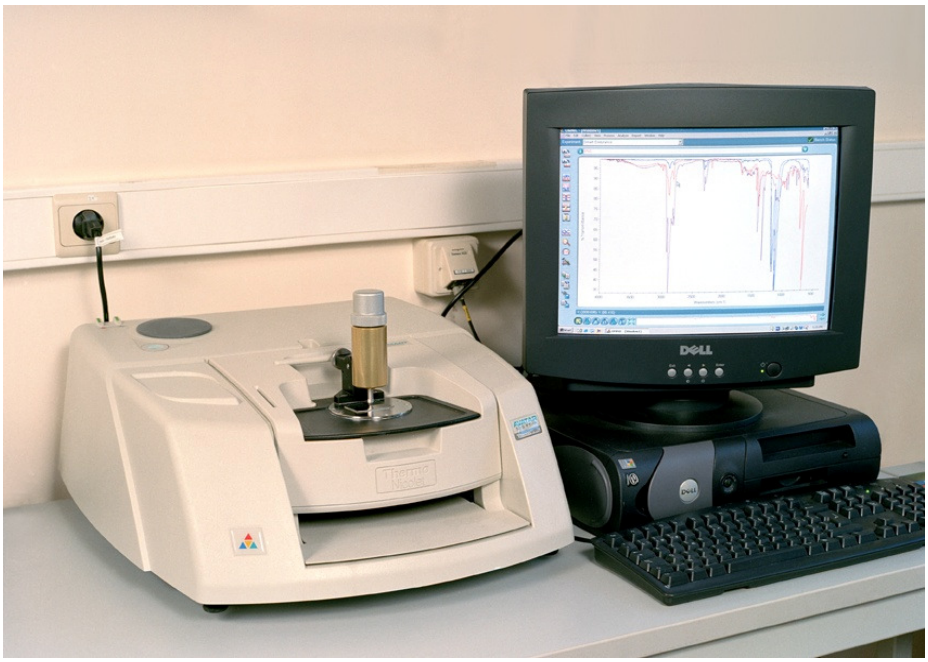



Fig No 4: FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)

**Introduction:**

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure .The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

**Principle:**

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

| | | |
|---|---|---|
| Source | : | Nernst Glower |
| Beam splitter | : | It is made up of a transparent material. Thin films of Silicon deposited on Potassium bromide (KBr) Bromide (KBr) Detectors: Deutrated TriGlycine Sulphate (DTGS). |
| Scan Range | : | MIR 450to 4000 cm-1 |
| Resolution | : | 4.0 cm-1 |
| Sample required | : | 50mg, solid or liquid |
| Sampling Techniques | : | There are a variety of techniques for sample preparation physical form of the sample to be analyzed. |
| Solid | : | KBr or Nujol mull method. |
| Liquid | : | Csl / TIBr Cells |
| Gas | : | Gas cells |

**Measurements Techniques:**

The procedure for recording the %T or %A is as follows:

Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.

Study of substances with strong absorbance bands and weak absorbance bands as well as possible.

Small amount of samples are sufficient

High resolution is obtained.

**Procedure:**

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

1. Then, 300 Al of 20 mM buffer, prepared in $H_20$ or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH
2. meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the this buffer.
3. The washings took 24 h, which is the time of contact of the protein with the 2H20
4. medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.
5. The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuterated triglycine sulfate detector.
6. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of $40^oC$. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in $5^oC$ steps from 20 to $95^oC$.
7. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of $H_20$ was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the $2H_20$ bending absorption close to 1220 cm_ 1

**KBr Method**

1. The sample is grounded using an agate mortar and pestle to give a very fine powder.
2. The finely powdered sample is then mixed with about 100mg dried KBr salt.
3. The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

**Nujol Mull Method:**

1. The sample is ground using an agate mortar and pestle to give a very fine powder.
2. A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
3. The plates are then placed in the instrument sample holder ready for scanning.

**Liquids:**

1. Viscous liquids can be smeared in the cell and directly measured.
2. For dilute solutions, liquid cells and variable path length cells are employed.

**Applications:**

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

## Mechanism of FTIR analyzer

### Analytical Capabilities:

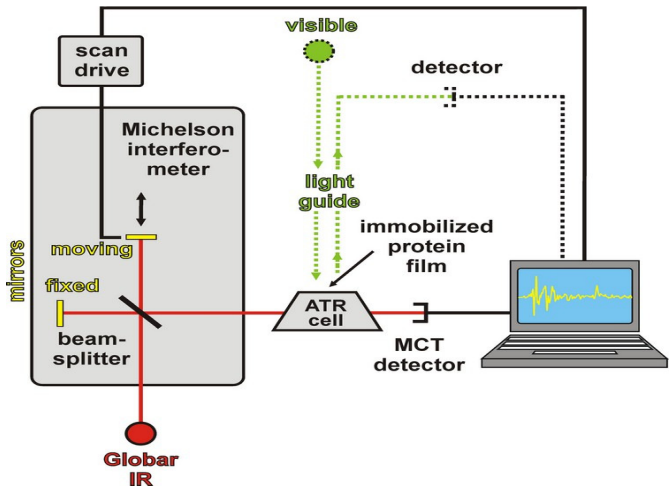



Fig No: 5Mechanism of FTIR

1. Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2. Especially capable of identifying the chemical bonds of organic materials
3. Detects and identifies organic contaminants.
4. Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5. Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm$^3$ or sometimes sub monolayer .Useful with solids, liquids, or gases.

# INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETER (ICP-OES)

Fig No: 6 ICP-OES

**Introduction:**

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

**Mechanism:**

The ICP-OES is composed of two parts: ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "work coil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that

creates a brief discharge arc through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. Stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colours), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colours fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for every element to which the unit is sensitive all at once. Thus, samples can be analysed very quickly.

The intensity of each line is then compared to previously measured intensities of known concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.

**Applications** :

ICP-OES is used in the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used it for elemental identification and quantitative compositional information of the *Komoothira silasathu parpam.*

# X-RAY POWDER DIFFRACTION

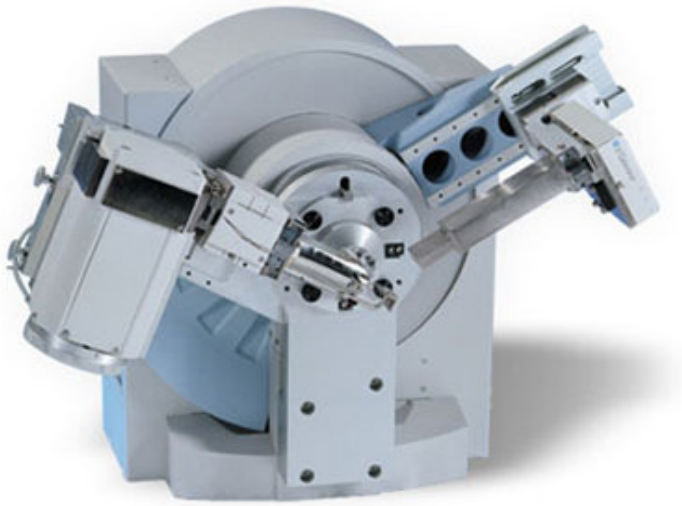


Fig-N0:7.X-RAY POWDER DIFFRACTION:

X-ray powder diffraction(XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions the analysed material is finely ground, homogenized, and average bulk composition is determined. Max von Laue, in 1912, discovered that crystalline substance act as three dimensional diffraction gratings for x-ray wavelengths similar to spacing of plants in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystaline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces concentrative interference(and diffracted ray) when conditions satisfy Brgg's Law(nX=2d sin9). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice sapcing in a crystalline sample.These diffracted X-rays are then detected, processed and counted. By scaning the sample through a range of 29 angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings. Typically, this is achived by comparsion of d-spacings with standard reference patterns.

All diffraction methods are based on generation of x-rays in a x-ray tube these x-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and differacted rays. Powder and single crystal diffraction vary in instrumentation beyond this.

X-ray powder diffraction(XRD) instrumentation

X-ray diffractometers consist of three basic elements: an X-rative, a sample holder,and an x-ray detector

X-rays are generated in a cathode ray tube by heating a filament toproduce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons havesufficiant energe to dislodge inner shell electrons of the target material,, characteristics X-ray spectra are produced.These spectra consist of several components, the most common being Ka and Kp. Ka consists, in part,of Ka1 and Ka2. Ka1 has, as lightly shorter wavelength and twice the intensity as Ka2. The specific wavelength are characteristic of the target material(Cu,Fe,Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X- rays needed for diffraction. Ka1 and Ka2 are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single – crystal diffraction, with CuKa radiation = 1.5418A.These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X- rays impinging the sample satisfies the Brgg Equation, constrictive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X- ray diffractometer is such that the sample rotates in the path of the collimated X- ray beam at an angle 9 while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 29. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 29 from -5$^{\circ}$ to 70$^{\circ}$, angle that are present in the X-ray scan

**Applications**

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of

unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

**Other application include:**

- Characterization of crystalline materials.
- Identification of fine grained minerals such as clays and mixed layer clays that are difficult to determine optically.
- Determination of unit cell dimensions.
- Measurement of sample purity.

**With specialized techniques,XRD can be used to:**

- Determine crystal structures using riveted refinement.
- Determine of modalamounts of minerals.

**Characterize thin film samples by:**

- Determine lattice mismatch between film and substrate and to inferring stress and strain .
- Determining dislocation density and quality of the flim by rocking curve measurements.
- Measuring super lattices in multi layered epitaxial structures.
- Determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements.
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

**Strength and limitation of X-ray power diffraction(XRD)**

- Powerful and rapid (<20 min ) technique for identification of an unkown mineral.
- In most cases, it provides an unambiguous mineral determination.
- Mineral sample preparation is required.
- XRD units are widely available.
- Data interpretation is relatively straight forward

**Limitations:**

- Homogenous and single phase material is best for identification of an unkown.
- Must have access to standard reference file of inorganic compounds (d-spacing,hkls)
- Requires tenths of a gram of material which must be ground into a powder.

- For mixed materials, detection limit is 2% of sample.
- For unit cell determination, indexing of patterns for non-isometric crystal systems is complicated.
- Peak overload may occur and worsens for high angle “reflection”
- Users guide – sample collection and preparation.
- Determination of an unknown requires the material, an instrument for grinding, and a sample holder.
- Obtain a few tenths of a gram (or more) of the material,as pure as possible grind the sample to a fine powder, typically in a fluid to minimize including extra strain (surface energy) that can offset peak position, and to randomized orientation. Powder less then $-10^2$ m (or 200mesh) in size is preferred.
- Place into a sample holder or on to the sample surface.
- Packing of fine powder into a sample holder.
- Smear uniformly on to a glass side, assuring a flat upper surface.
- Pack into a sample container
- Sprinkle on double sticky tape.
- Typically the substrate is amorphous to avoid interference.
- Care must be taken to create a flat upper surface and to archive a random distribution of lattice orientations unless creating an oriented smear.
- For analysis of clays which require a single orientation, specialized techniques for preparation of clay samples are given by usages,
- For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak position.

**Data collection, Results and Presentation**:

**Data Collection:**

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d - spacing appropriate to diffract X-rays at that value of 9. Although each peak consists of two separate reflections ( ka1 and ka2), at small values of 29 the peak locations overlap with ka2 appearing as a hump on the side of ka1. Greater separation occi=urs at higher values of 9. Typically these

combined peaks are treated as one. The 21 position of the diffraction peak is typically measured as the center of the peak at 80%peak height.

**Data Reduction:**

Results are commonly presented as peak positions at 29 and X-ray counts(intensity) in the form of an x-y plot. Intensity (I) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak ( relative intensity = 1/11×100).

**Determination of an unknown:**

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of 1. Once all d-spacing have been determined, automated search match routines compare the d-spacing of the unknown to those of unknown materials. Because each mineral has a unique set of d-spacing, matching these d-spacing provides an identification of the unknown sample. A systemic procedure is used by entering the d-spacing in terms of their intensity beginning with the most intense peak. Files of d-spacing for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction file ( PDF). Many other sites contain d-spacing of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

# 4.3. PHARMOCOLOGICAL STUDIES

## 4.3.1. HYPOGLYCEMIC ACTIVITY OF *KOMOOTHIRA SILASATHU PARPAM* IN STREPTOZOTOCIN INDUCED WISTAR ALBINO RATS(IN VIVO STUDY):

**EXPERIMENTAL MODELS**

For the study of anti-diabetic an experimental model is selected in such a way that it would satisfy the following:

The animal should develop hyperglycemia rapidly.

Pathological changes in the site of induction should result from pancreatitis or damage of β-cells.

The symptoms should be ameliorated or prevented by a drug treatment effective in human beings.

**Materials:**

Animals :Male albino wistar rats (180-220gm)

Drugs : Powder of ***KSP***

Chemical: STREPTOZOTOCIN(SPZ) (S. D Fine. Chem. Ltd, Mumbai)

**Selection & acclimatization of animals:**

Wistar strains of male albino rats weighing between 180-220gm are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature ($22^0$c ± $5^0$c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

**INDUCTION OF DIABETES MELLITUS**

Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of ***SPZ*** (150mg/kg BW) in physiological saline after overnight fasting for 12hrs.[1]

***SPZ***is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as $H_20_2$, $0_2$ and $H_2$ .The development of hyperglycemiain rats is confirmed by plasma glucose estimation 72 hrs post ***SPZ*** injection. The rats with fasting plasma glucose level of 160-220mg/dl were used for this experiment.

**Experimental procedure:**

In the experiment a total of 30 rats (24 diabetic surviving rats & 6 normal rats) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 6 groups after the induction of ***SPZ*** diabetes. In the experiment 6 rats were used in each group.

**TREATMENT PROTOCOL:**

| | | |
|---|---|---|
| Group-I | : | (Normal control) consist of normal rats given with 10ml/Kg of normal saline, orally. |
| Group-II | : | (Toxic control) Diabetic control received 150mg/Kg of Streptozotocin through I.P. |
| Group-III | : | Diabetic control receivedglipizide at a dose of (10mg/Kg orally) for 28 days. |
| Group-IV | : | Diabetic control received powder of***KSP***at a dose of (200mg/Kg orally) for 28 days. |
| Group-V | : | Diabetic control received powder of ***KSP*** at a dose of (400mg/Kg orally) for 28 days. |

**METHODOLOGY:**

**Sample collection:**

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocularpuncture in EDTA coating plasma tubes for the estimation of blood parameters.

**BIOCHEMICAL ANALYSIS:**

**Estimation of blood glucose:**

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson based on glucose oxidase method.

**Plasma insulin:**

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit with an ES300 Boehringeranalyzer (Mannheim, Germany).

**Estimation of total haemoglobin and glycosylated haemoglobin:**

Total haemoglobin was determined by the method of Drabkin and Austin (1932) and glycosylated haemoglobin was determined by the method of SudhakarNayak and Pattabiraman (1981).

**Estimation of lipid & lipoprotein:**

Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970) (total cholesterol), Gidez and Webb (1950) (HDL-cholesterol), Zilversmith and Davis (1950) (phospholipids) and Rice (1970) (triglycerides).

**Statistical analysis:**

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at $p<0.01$.

## 4.3.2 ANTI DYSLIPIDEMIC ACTIVITY OF *KOMOOTHIRA SILASATHU PARPAM* in Wistar Albino Rats (in Vivo study)

**ANTI DYSLIPIDEMIC MODELS OF WISTAR ALBINO RATS (IN VIVO STUDY):**

**Animals**

Wistar albino adult male rats weighing 150-200gm from animal housing facility of Vels University were housed in polypropylene cages maintained with temperature 27°C ±1°C and 12 hrs light and dark cycles. The animals were allowed to adapt to the environment for seven days and supplied with a standard pellet diet (Sai Durga foods, Bangalore) and water *ad libitum*. The experimental protocol has got the approval IAEC bearing no

**PharmacologicalEvaluation**

All animals starved for 18 hours and provided water *ad libitum* before the experiment. The animals were divided into five groups of six rats each. Group I served as normal control administered with 2% CMC only. Group II served as anti dyslipidemic control given a single dose of triton was administered 400 mg/kg only. Group III and IV served as test groups received KC 250mg/kg and KC 500mg/kg respectively. Group V served as Lovastatin (10mg/kg/day) considered as standard. All the groups except the normal control group administered a single dose of Triton dissolved in 0.9% Normal saline intraperitonially. After inducing the hyperlipidemia, the respective treatment was continued for 7 days. Animals were given standard pellet diet and water *adlibitum.*

**Collection ofblood**

The next day after the completion of experimental study, the blood was taken from the rats under mild anesthetic state by retro orbital sinus puncture. The collected blood samples were centrifuged (2500 rpm) for 10 minutes. Then serum samples were separated and it was used for various biochemical analyses. Then animals were sacrificed and the liver, heart and kidney were taken for histopathological study and for the analysis of organ weight.

**Liver lipid extraction**

The liver was homogenized in cold 0.15M KCl and extracted with CHCl3: CH3OH (2% *v/v*). This lipid extract was used for the estimation of lipid parameters.

**Biochemical analysis**

The serum and liver were analyzed for serum total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) by standard enzymatic calorimetric methods.

**Histopathology**

All rats were sacrificed after the collection of blood sample. Liver was excised from the rats to visually detect gross lesions, and weighed to determine weight variation and preserved in 10% neutral formalin for histopathological assessment. The tissue was embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

## 4.3.3 INVITRO ANTIOXIDANT ACTIVITY KOMOOTHIRA SILASATHU PARPAM ON DPPH (2,2-DIPHENYL-1-PICRYL HYDRAZYL):RADICAL SCAVANGING METHOD:

The antioxidant activity of *KSP* was determined using the 2, 2-diphenyl-1 picrylhydrazyl (DPPH) free radical scavenging assay[70].100µl of *KSP* extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates[71,72].

Free radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs of Control} - \text{Abs of Test})}{\text{Abs of Control}} \times 100$$

# 4.4 MICROBIOLOGICAL ANALYSIS

Antimicrobial activity was performed by agar well diffusion method on agar.

**Preparation of drug extracts solutions for the experiment**

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

**Procedure for the Agar Well Diffusion Test**

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

**Bacterial Inoculums Preparation**

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

**Agar well-diffusion method**

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

**Composition of Muller Hinton Agar Media**

| | |
|---|---|
| Beef Extract | : 02.00 gm |
| Acid Hydrolysate of Casein | : 17.50 gm |
| Starch | : 01.50 gm |
| Agar | : 17.00 gm |

# 4.5. TOXICOLOGICAL STUDIES

## 4.5.1. ACUTE TOXICITY STUDY OF *KOMOOTHIRA SILASATHU PARPAM* IN FEMALE WISTAR ALBINO RATS

### OBJECTIVES:

The aim of this Study is to evaluate the toxicity of the test medicine *komoothira silasathu parpam*, when administered orally to Female Wistar Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

**GUIDELINES FOLLOWED:**

OECD Guidelines No. 423.

**STUDY DESIGN AND CONTROLS:**

1. Female WISTAR Rats in controlled age and body weight were selected.
2. The test drug *Komoothira silasathu parpam* was administered at 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg, and 2000mg/kg body weight of animal as suspension along with water.
3. The results were recorded on day 0, with single oral dosing and observed for a period of 14 days.

**EXPERIMENTAL PROCEDURE**

**1.ANIMALS**

**1.1 . Supply:**

A total of 15 Female wistar Rats with an approximate age of 6 weeks and purchased from Tiruvandrum medical college, Tiruvandrum. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female WISTAR Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

**1.2.Housing:**

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the StudyThe Female wistar Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

**2.DIET:**

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

**2.1. Water:**

The water was offered adlibitum in bottles.

**3. NUMBERING AND IDENTIFICATION:**

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

**Table no-5 Acute Toxicity Numbering and Identification of Animals**

| Group No | Animal Marking |
|---|---|
| 1 | Head |
| 2 | Body |
| 3 | Tail |

**Table no 6.Numbering and Identification of Animal**

| Cage No | Group No | Animal Marking | Sex |
|---|---|---|---|
| 1 | I | H,B,T | Female |
| 2 | II | H,B,T | Female |
| 3 | III | H,B,T | Female |
| 4 | IV | H,B,T | Female |
| 5 | V | H,B,T | Female |

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

**3. ADMINISTRATION ROUTE AND PROCEDURE:**

The test substance was administered orally. The Female WISTAR Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

**3.1. Doses**

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

**Table no 7. Doses**

| GROUP | DOSE |
|---|---|
| **GROUP** | **DOSE** |
| **Group-I** | **5 mg/kg** |
| **Group-II** | **50 mg/kg** |
| **Group-III** | **300 mg/kg** |
| **Group-IV** | **1000 mg/kg** |
| **Group-V** | **2000 mg/kg** |

The test drug was administered as single dose. After single dose administration period, all animals were observed for 14days.

**DOSE PREPARATION:**

*Komoothira silasathu parpam* was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

**3.2.ADMINISTRATION:**

The test medicine was administered orally to each Female WISTAR rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

### 3.3. OBSERVATION PERIOD:

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

### 3.4.MORTALITY AND MORBIDITY:

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days**.**

## 4.5.2 SUB-ACUTE(Repeated Dose) TOXICITY STUDY OF *KOMOOTHIRA SILASATHU PARPAM* IN WISTAR ALBINO RATS

**1. OBJECTIVE**

The objective of this 'Sub-Acute Toxicity Study of *komoothira silasathu parpam* WISTAR Rats' was to assess the toxicological profile of the test item when treated as a single dose daily. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

**2.TEST GUIDELINE FOLLOWED**

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

**3. TEST ITEM DETAIL**

*Komoothira silasathu parpam*

**4.TEST SYSTEM DETAIL**

The study was conducted on 5 male 5 female WISTAR rats for each group. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at Animal house, KLU college of pharmacy, Madurai. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

**5. ACCLIMATIZATION**

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

**6. RANDOMIZATION& GROUPING**

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 female animals per group.

**7.NUMBERING AND IDENTIFICATION**

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

**Table No:8**

| Cage No | Group no & Concentration/Dose mg/kg/day | Animal Marking | Sex | No of rats |
|---|---|---|---|---|
| 1 | 1. Control | H,B,T,HB,NM<br>H,B,T,HB, NM | Male<br>Female | 5<br>5 |
| 2 | 2. Low dose of KSP(200mg/kg) | H,B,T,HB,NM<br>H,B,T,HB, NM | Male<br>Female | 5<br>5 |
| 3 | 3.Middledose of KSP(400mg/kg) | H,B,T,HB,NM<br>H,B,T,HB, NM | Male<br>Female | 5<br>5 |
| 4 | 4. High dose of KSP(600 mg/kg) | H,B,T,HB,NM<br>H,B,T,HB, NM | Male<br>Female | 5<br>5 |

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the Above

**8. HUSBANDRY**

**8.1 HOUSING:**

The WISTAR rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 10 rat of the differnt sex and treatment group.

**8.2 ENVIRONMENTAL CONDITIONS:**

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at $22\pm3^{0}$C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

**8.3 FEED & FEEDING SCHEDULE:**

'Sai Durga Animal Feed, Bangalore. Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

**8.4 WATER:**

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

**8.5 DOSES:**

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

The test item was administered as single dose daily. After single dose administration period, all animals were observed for 28 days.

**DOSE PREPARATION**

*Komoothira silasathu parpam* was added in distilled water and completely dissolved for oral administration. The dose was prepared of a required concentration before dosing by dissolving *komoothira silasathu parpam* in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

**8.6 ADMINISTRATION**

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

**9. OBSERVATIONS**

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

**9.1. CLINICAL SIGNS OF TOXICITY**

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

**9.2. FOOD INTAKE**

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

**9.3. WATER INTAKE**

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

**9.4 BODYWEIGHT:**

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

**Blood Collection** :

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

**LABORATORY STUDIES**

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, PLATELETS, MCH, PCV, Hb. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP, BILIRUBIN, CREATININE, URIC ACID and Electrolytes like Sodium, Calcium, phosphorus.

**TERMINAL STUDIES**

**Sacrifice and macroscopic examination**

On completion of the 4 weeks of treatment, 18 WISTAR rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

**Organ weights:**

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

# 5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the trial drug *Komoothira silasathu parpam.*(KSP) The drug KSP**.** has been selected for Hypoglycemic, Anti dyslipidemic, & anti oxidant activity in reference with the text **"***Thanjai Vaithiya Raja Sinthamani"* Part – 2 Page No 5. The study includes Literary collections, Organoleptic character, Physicochemical and Instrumental analysis, and Pharmacological studies. Toxicological studies

Literature collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating diabetes mellitus.(*madhumegam*).

Botancial aspects explains the identification, description, active principle and medicinal uses of the palnts.

Gunapadam review brings the effectiveness of the drug in treating diabetes mellitus.

Modern and siddha aspect of the disease was also reviewed.

Pharmaceutical review describes about the *Parpam* and its properties.

The pharmacological study explains about the methodology of Hypoglycemic,

Anti dyslipidemic, & Anti- oxident activites

**STANDARDIZATION OF THE TEST DRUG**

Standardizations of the drug is more essential to derive the efficacy and potency of the drug, which was analysed by the various methods. The results of physicochemical and biochemical analysis have been done and tabulated. Pharmacological activity and toxicological results of the drug were derived. The result reveals the effectiveness of the trial drug *KSP* has been proved by the following scientific parameters.

**PHYSICO CHEMICAL ANALYSIS**

The following characters have been noted in *KSP*.

**Table No -9.organoleptic character**

| | |
|---|---|
| Colour in day light | White |
| Odour | Pleasant odour |
| Taste | Astringent |
| Appearance | Fine powder |
| Sense of Touch | Fine |

**Table No –10.Physicochemical properties**

| Sl. No | Parameters | Values | Normal Values |
|---|---|---|---|
| 1 | Water soluble ash | 7.65±0.011 | 7.85 % |
| 2 | Acid insoluble ash | 0.85±0.011 | 7.45% |
| 3 | Loss on drying at 70°C | 7.10±0.240 | 5 – 8 % |
| 4 | pH Analysis | 8.540 | > 7 % |

# INTERPRETATION:

**Organoleptic character:**

Organoleptic character indicates that the test drug *KSP* has the following characters, fine powder, White in colour, Pleasant odour, astringent in taste, fine to touch.

**Loss on drying:**

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The percentage loss on drying 7.0% was within acceptable range (5%-8%),

thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

**Ash value:**

**Determination of ash value:**

The Ash Limit Tests are designed to measure the amount of the residual. Substances when a sample is ignited under the conditions specified in the individual monograph. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug. The total ash values of KSP were 68.2% .The value of total ash in the formulation is comparatively high. The value of total ash indicates that the inorganic contents of the formulation are below the limits. This signifies the ash value determination as an important parameter to standardize the herbal drugs.

**The Acid-Insoluble Ash Limit Test is designed to measure the amount of ash:**

Insoluble to diluted hydrochloric acid.Acid-insoluble ash value of the prepared formulation (22.08%) shows that a very small amount of the inorganic component is insoluble in acid.It indicates that adulteration of raw ingredients by substances, such as silica and rice husk, is very less, and a low acid-insoluble ash value may also affect the amount of the component absorbed in the gastrointestinal canal when taken orally.

**Determination of Alcohol-soluble and water-soluble extractive values**

Alcohol-soluble and water-soluble extractive values of ingredients and formulation are depicted in table which shows 81.2% alcohol-soluble extractive value and 97.7% water-soluble extractive value of the formulation. Higher water-soluble extractive value implies that water is a better solvent of extraction for the formulation than ethanol. acid-insoluble ash, and water-soluble ash were found to be 68%,22.08%,71.2% respectively; the value of total ash indicates that the inorganic contents of the formulation are below the limits. The results of Alcoholic and water soluble extracts of the formulation show that alkaloids of the formulations are more soluble in water than alcohol and a higher water soluble extractive value of the formulation depicts that water is a better solvent of extraction for the formulation than alcohol.

# MICROBIAL LIMIT TESTS

**Table No: 11. Results for Microbial limit test :**

| S.No | Microbes | Colony measurements | Normal limits |
|---|---|---|---|
| 1. | Total viable aerobic count | 4 x$10^4$ col/g | 1 x$10^5$ col/g |
| 2. | Total fungal count | Nil | 1 x$10^4$ col/g |
| | **Test for specific pathogen** | | |
| 1. | *Salmonella sp* | Nil | Nil |
| 2. | *Staphylococcus aureas* | Nil | Nil |
| 3. | *E.coli* | Nil | Nil |
| 4. | *Pseudomonas aeruginosa* | Nil | Nil |

Total viable aerobic counts within the normal level.

Total fungal count within the normal level.

Specific pathogens like *Salmonella sp., Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa* are Nil.

Hence, the test drug is free from any microbial contamination.

## BIO-CHEMICAL ANALYSIS OF *KOMOOTHIRA SILASATHU PARPAM*

**Table No:12. Result of Preliminary Basic and Acidic Radicals studies**

| S.No | Test | Inference |
|---|---|---|
| 1 | Test for calcium | Present |
| 2 | Test for sulphate | Present |
| 3 | Test for chloride | Present |
| 4 | Test for carbonate | Absent |
| 5 | Test for starch | Absent |
| 6 | Test for Iron ferric | Absent |
| 7 | Test for iron ferrous | Present |
| 8 | Test for phosphate | Absent |
| 9 | Test for Albumin | Absent |
| 10 | Test for Tannic acid | Absent |
| 11 | Test for unsaturation | Present |
| 12 | Test for the reducing sugar | Absent |
| 13 | Test for amino acid | Present |
| 14 | Test for zinc | Absent |

## INTERPRETATION:

From the test for basic and acidic radicals studies shows the presence of

1.Calcium,

2.sulphate,

3.Chloride.

4.Ferrous Iron.

5.Amino acid

**1.Calcium:**

It is an essential component of intracellular process that occur within insulin responsive tissues like skeletal muscle and adipose tissue. A very narrow range of calcium concentration is needed for optimal insulin mediated functioning.

Concentrated levels of calcium out of optimal range may contribute to peripheral insulin resistance.

**2.Sulphates:**

Sulphate has anti bacterial activity and it is one of the macronutrient of cells.

Sulphate important role for the anti – microbial activity.

It is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases, food is not digested efficiently.

**3.Chloride :**

Chloride regulates the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids.

**4.Ferrous Iron :**

Iron is easily soluble and readily absorbed from intestine and involved.

**5.Amino acid:**

In diabetes mellitus atherosclerotic changes occur earlier ,Amino acids, like arginine, help the arteries of the body retain elasticity, which prevents them from stretching out and allowing fluid to collect. They also help support the expansion and contraction of the arteries with each heartbeat.

The tissues in the body -- muscles, skin, connective tissues, etc. -- need amino acids for repair when injured or damaged. They're especially beneficial after exercise or difficult training, when the muscles can tear. Taking amino acids like arginine after exercise can help the muscles recover and heal properly and more quickly.

## INSTRUMENTAL ANALYSIS

## SEM (SCANNING ELECTRON MICROSCOPE) RESULT:

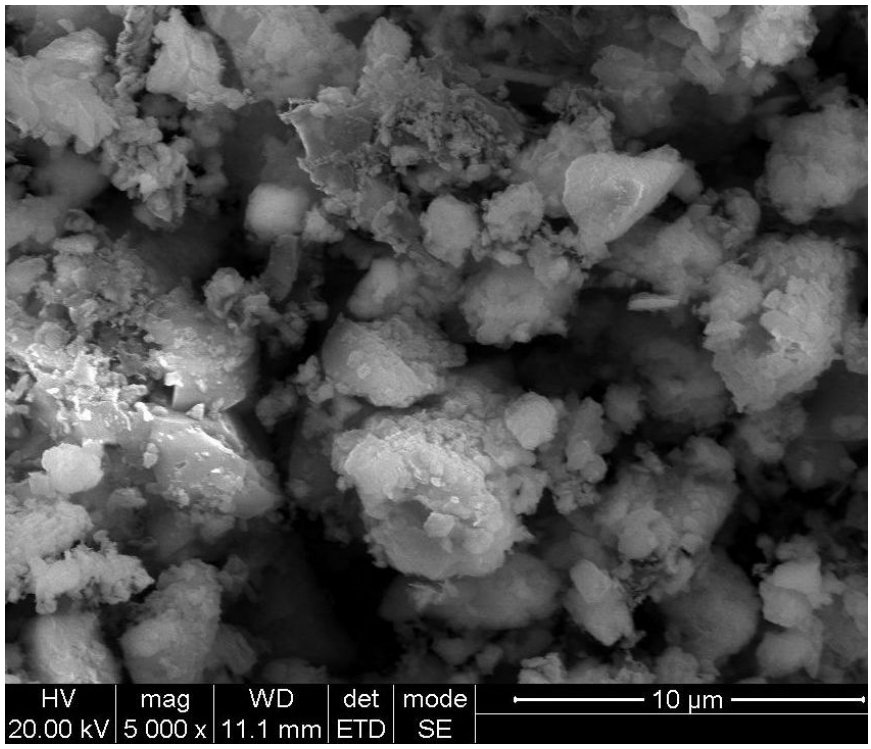



Fig.No.8. SEM Picture 30,000 Magnification.

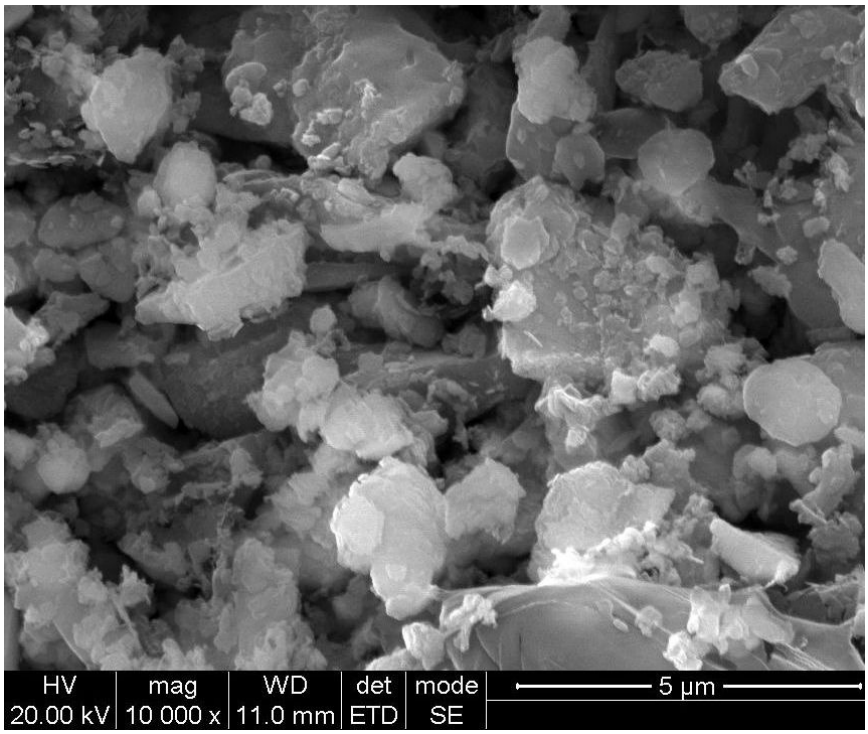



Fig.No.9. SEM Picture 80,000 Magnification.

SEM analysis of the *KSP* shows the particle size varies between 5µm to 10µm. The surfaces of the sample grains is uniformly arranged in agglomerates.

Smaller sized particles enhance the absorption and bio availability resulting efficacy of the drug will be increased.

**The microparticles**

Increase drug therapeutic efficacy

Increase bio-availability

Reduces side effect

In SEM analysis the micro particle present in the test drug results in a better bioavailability and facilitates absorption.So very minimal quantity of the medicine is enough to treat the disease, resulting better efficacy of the drug. Larger particles could not enter in to the target cell because of their size, and it will requires extra digestive and absorptive load, resulting they easily cleared from the blood

## FT-IR RESULTS OF *KSP:*

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material. In FTIR the wave numbers between $4000^{cm-1}$ – $600^{cm-1}$ is known as functional group area. $<600^{cm-1}$ wave numbers is known as fingerprint area. The corresponding absorption frequency by FTIR shows the presence of alcohol, phenol, $1^0$, $2^0$amines, amides, alkanes, $1^0$ amines, aromatics, aliphatic amines and alkyl halides are present in *KSP*

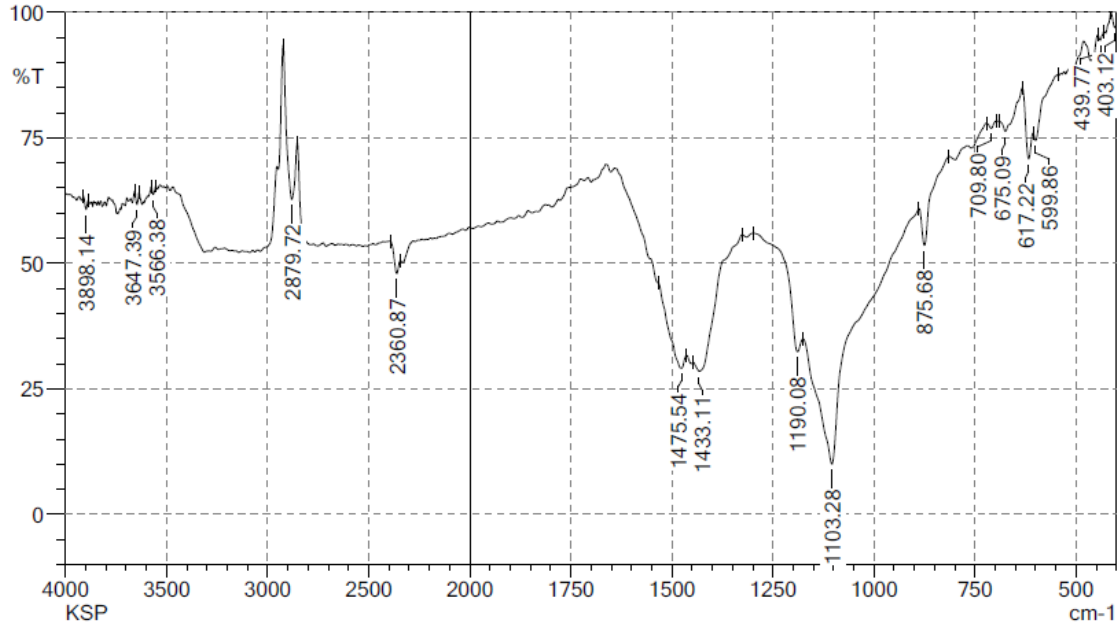



**Fig: no: 10. FTIR image of *KSP***

FTIR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. *KSP* has the following functional groups.

## Table 13: FTIR Spectrum- functional groups

| Frequency ($cm^{-1}$) | Bond | Functional Groups |
|---|---|---|
| **701.80** | **C = C Bending** | **Alkane** |
| **885.68** | **C = C Bending** | **Alkane** |
| **1475.54** | **C – H bonding** | **Alkcane** |
| **2360.87** | **O = C = O steching** | **Carbondioxide** |
| **2879.72** | **C – H streching** | **Aldehyde** |
| **3566.38** | **N – H Streching** | **Primary Amine** |
| **3647.39** | **O – H streching** | **Alchols.** |

The test drug was identified to have 10 peaks which represent the functional groups present in the test drug, *KSP*

**Alkanes groups:**

Alkanes have little biological activity.

It is predominate in plants. They protect against bacteria and fungi.

**Alcohols**:

Alcholic group of substance act as anti microbial and antiseptic agents.

**Amines:**

Amine groups act as neurotransmitters

It is involved in protein synthesis.

This group of substance has antihistaminic and analgesic activity.

**Aldehyde:**

It acts as blood purifier.

It effects free radicals from the blood.

## ICP-OES of *Komoothira Silasathu Parpam*

**ICP-OES of *KSP***

**Table No. 14.**

| Sl.No | Elements | Wavelength (nm) | Concentration |
|---|---|---|---|
| 1 | Al | 396.152 | BDL |
| 2 | As | 188.979 | BDL |
| 3 | Ca | 315.807 | 821.170 mg/L |
| 4 | Cd | 228.802 | BDL |
| 5 | Cu | 327.393 | BDL |
| 6 | Fe | 238.204 | BDL |
| 7 | Hg | 253.652 | BDL |
| 8 | K | 766.491 | 03.071 mg/L |
| 9 | Mg | 285.213 | 01.104 mg/L |
| 10 | Na | 589.592 | BDL |
| 11 | Ni | 231.604 | BDL |
| 12 | Pb | 220.353 | BDL |
| 13 | P | 213.617 | 176.307 mg/L |
| 14 | S | 180.731 | 501.254 mg/L |

**BDL: Below Detectable Limit(Normal-1ppm)**

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

**Table No 15.Toxic metals and the permissible limits**

| Heavy metals | WHO & FDA limits |
|---|---|
| Pottasium (K) | 25.621 mg/L |
| Sodium (Na) | 24.310 mg/L |
| Phosporus (P) | 126.341 mg/L |

## INTERPRETATION:

ICP-OES reveals high concentration of Sulphate in *KSP* (0.50120 mg/L). It also has physiologically important minerals like Calcium, Phosphorus, megnesium, sulfer, sodium and Potassium. In *KSP ,* the heavy metals like Arsenic, Mercury, Lead, Cadmium and trace element like nikkal were below detectable level. This reveals the safety of the drug and it has free from toxic substances and has no side effects.

**Sodium:**

Sodium regulates the acid-base balance of the body fluids.

Sodium is required for the maintenance of osmotic pressure of the body fluids.

It is necessary for the normal muscle irritability and permeability of cells.

Sodium maintains extracellular osmotic pressure.

**Potassium:**

Potassium is required for the regulation of acid-base balance and water balance of the body fluids.

Potassium maintains intracellular osmotic pressure.

Potassium is required for the transmission of nerve impulse.

**Calcium:**

Calcium influences the membrane structure and transport of water and several ions across it.

The release of certain hormones (insulin, PIH, calcitonin) from the endocrine glands is facilitated by $ca^{2+}$

**Sulphur:**

Early research suggests that drinking water from a sulfurous spring three times daily for 4 weeks reduces total cholesterol,low- density lipoprotein (LDL or Bad) cholestrol, and triglyceride levels. However ,it's not clear from this study alone if sulfur might reduce cholestrol.

**Magnesium:**

Eating a diet with more magnesium is linked with a reduced risk of developing diabetes in adults and overweight children. research on the effects of magnesium for people with existing type 2 diabetes shows conflicting results.

**Phosphorus :**

Phosphorus is important for energy transfer in cells as part of ATP and is found in many other biological important molecules. Thus it relieves fatigue.

# X- RAY POWDER DIFFRACTION:

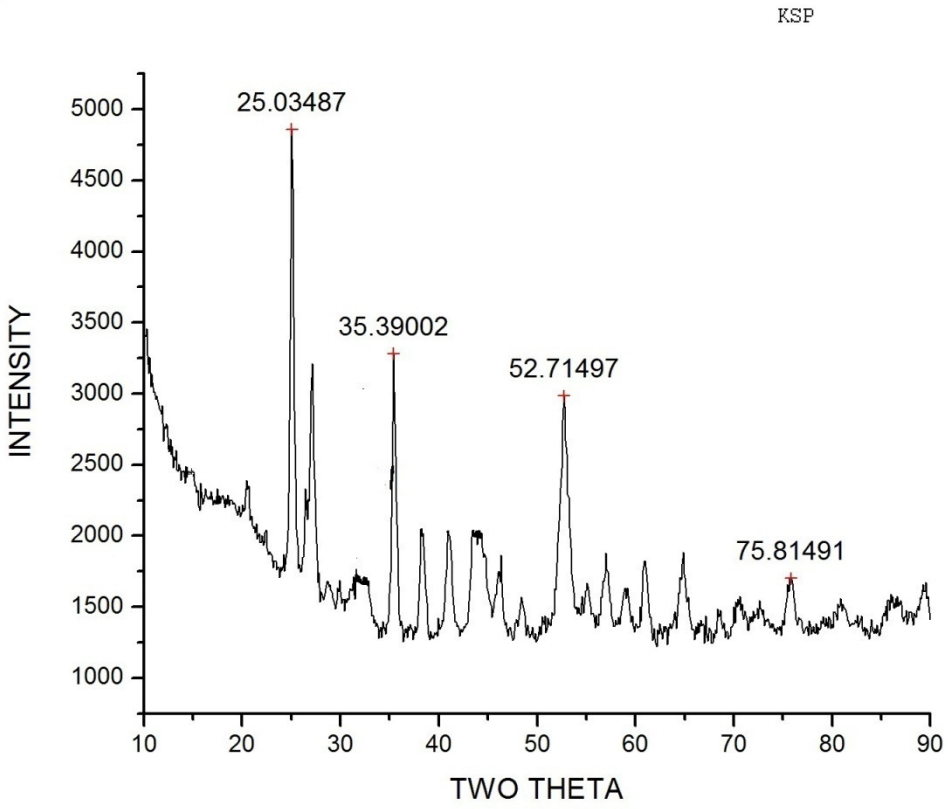



**Fig No:11.XRD IMAGE OF *KSP***

**Interpretation:**

These XRD fingerprints shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo mineral medicines. Mordern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature. The different peaks shows the presence of minerals in the sample.

# PHARMACOLOGY STUDY

## HYPOGLYCEMIC ACTIVITY OF KSP IN STREPTOZOTOCIN INDUCED IN WISTAR ALBINO RAT IN VIVO STUDY

| GROUP No | Body weight (g) | | Blood glucose (mg / 100ml) | Blood glucose (mg / 100ml) |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| **G1 Normal** | 122 ± 7.30 | 135 ± 7.60 | 84.65 ± 3.30 | 87.70 ± 3.75 |
| **G2 Diabetic** | 120 ± 6.70 | 172 ± 4.50**[(a)] | 90.75 ± 3.85 | 210.45 ± 6.70**[(a)] |
| **G3 Standard** | 130 ± 7.50 | 125 ± 7.25 | 87.55 ± 4.22 | 118.30 ± 4.42**[(b)] |
| **G4 Low dose** | 122 ± 7.25 | 140 ± 8.10 | 84.80 ± 3.60 | 135.45 ± 5.20**[(b)] |
| **G5 High dose** | 130 ± 7.30 | 132 ± 7.40 | 86.50 ± 3.80 | 124.40 ± 4.65**[(b)] |

**Table No: 16 Effect of *KSP* on initial and final body weight and blood glucose in normal and treated animals.**

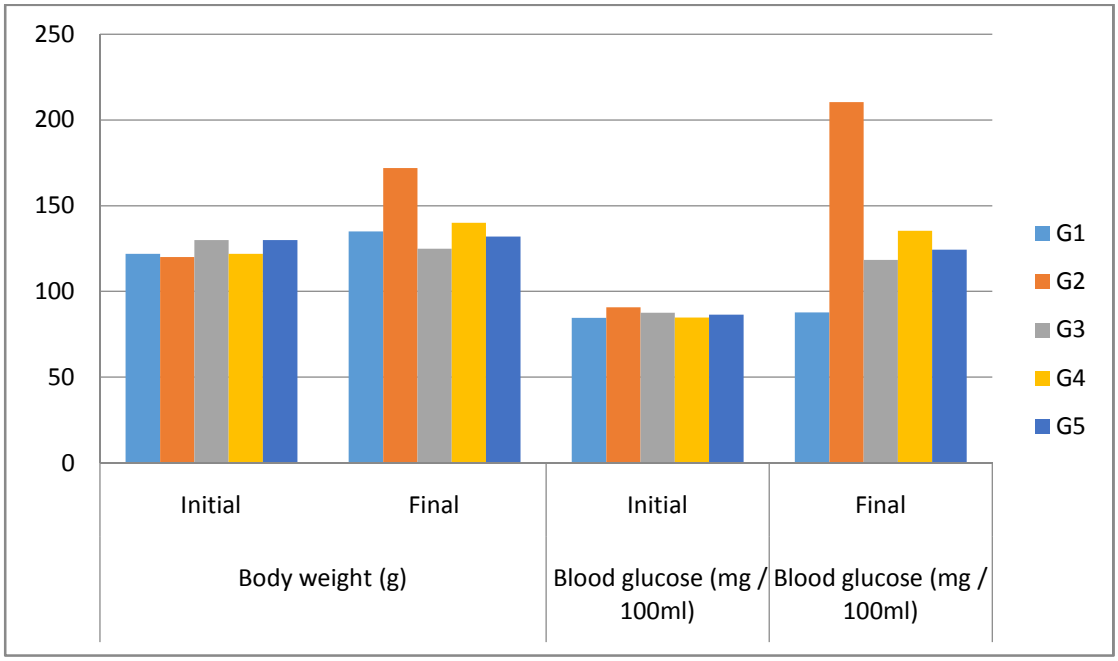



**Fig No: 12 Effect of *KSP*on initial and final body weight and blood glucose in normal and treated animals.**

Values are expressed as mean **±** SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

(a) Values are significantly different from normal control G1 at P<0.001.

(b) Values are significantly different from Diabetic control G2 at P<0.01.

**Table no:17 -EFFECT OF *KSP* ON PLASMA INSULIN, HEMOGLOBIN& GLYCOSYLATED HEMOGLOBIN ($HbA_1C$) IN NORMAL AND TREATED ANIMALS.**

| GROUPS | Haemoglobin (gm/100ml) | Glycosylated haemoglobin[$HbA_1C$(%)] | Plasma Insulin (µU/ml) |
|---|---|---|---|
| **G1** | 12.80 ± 1.60 | 0.38 ± 0.06 | 33.50 ± 2.90 |
| **G2** | 7.10 ± 0.70**[(a)] | 0.89 ± 0.14**[(a)] | 10.60 ± 1.70**[(a)] |
| **G3** | 11.10 ± 1.22**[(b)] | 0.34 ± 0.06**[(b)] | 27.45 ± 2.40**[(b)] |
| **G4** | 10.30 ± 0.92**[(b)] | 0.43 ± 0.05**[(b)] | 27.60 ±2.30**[(b)] |
| **G5** | 10.96 ± 1.12**[(b)] | 0.42 ± 0.09**[(b)] | 28.95 ± 2.35**[(b)] |

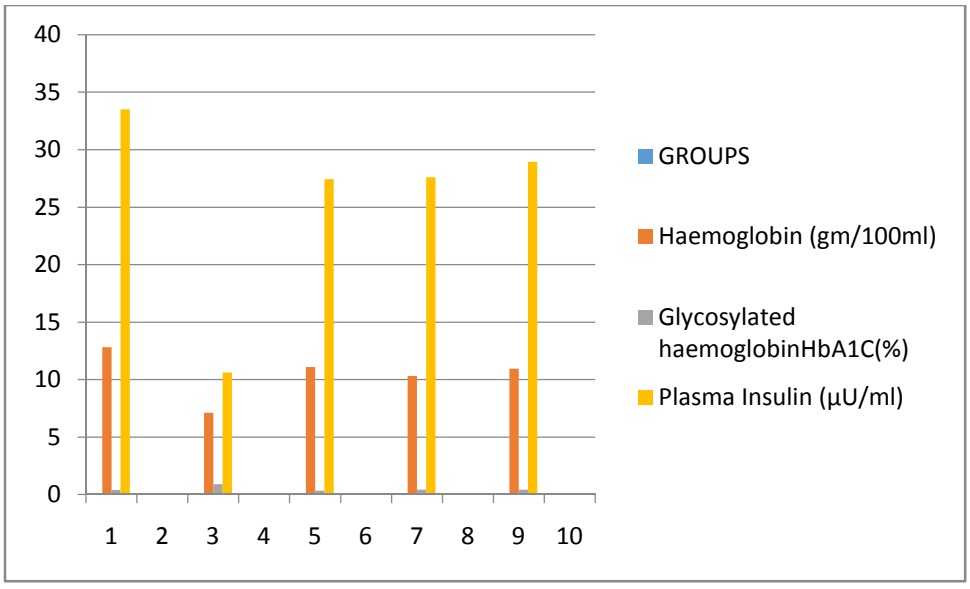



**Fig No 13 -Effect of *KSP* on plasma insulin, Hemoglobin& Glycosylated hemoglobin in normal and treated animals.**

Values are expressed as mean **±** SEM.

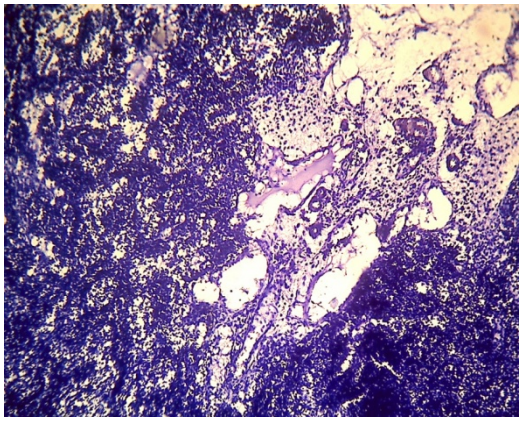
Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

(a) Values are significantly different from normal control G1 at P<0.001.

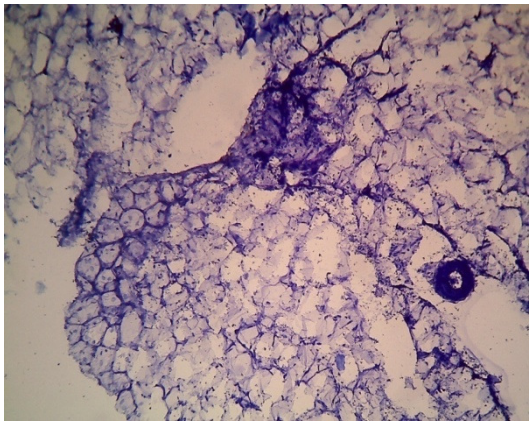
(b) Values are significantly different from Diabetic control G2 at P<0.01.

**Fig No :14.**

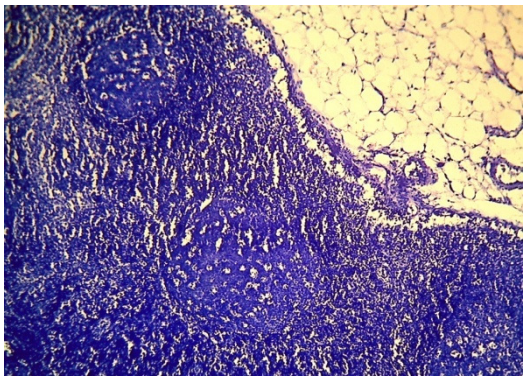
**HYPOGLYCEMIC– NORMAL**



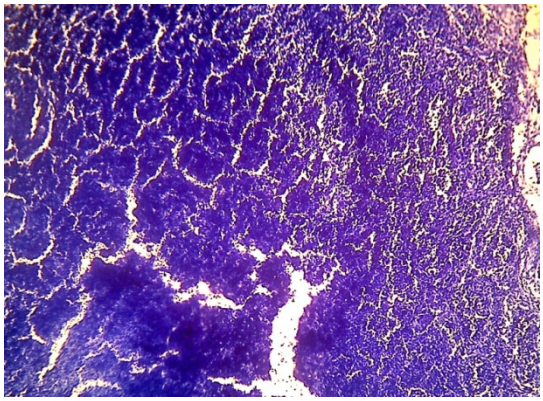
**SPZ INDUCEED**



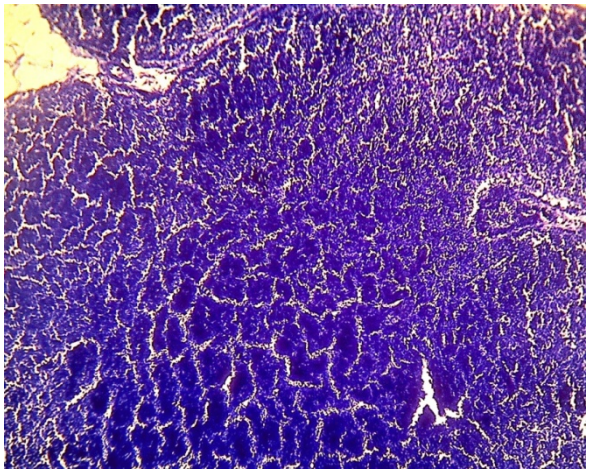
**HYPOGLYCEMIC – STD/CONTROL**



**HYPOGLYCEMIC – LOW DOSE**



**HYPOGLYCEMIC – HIGH DOSE**



**RESULT:**

**INTERPRETATION**:

**BLOOD GLUCOSE LEVEL:**

SPZ causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. The mechanism of SPZ action was fully described elsewhere (Lazarow, 1964; Colca et al., 1983). In our study, we have observed a significant increase in the plasma insulin level when SPZ induced diabetic rats were treated with *KSP* at a dose of 200mg/kg and 400mg/kgthis could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β- cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens (Alberti and Press, 1982). Glycosylated haemoglobin ($HbA_1C$) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) and the amount of increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979). During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in SPZ induced diabetic rats (Sheela and Augusti, 1992). Administration of *KSP* at a dose of 200mg/kg and 400mg/kg for 28 days prevents a significant elevation in glycosylated haemoglobin there by increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by *KSP* at a dose of 200 mg/kg and 400 mg/kg.

The body weight was decreased in SPZ diabetic rats.Administration of *KSP* at a dose of 200mg/kg and 400mg/kg increases the body weight in SPZ induced diabetic rats. The ability of *KSP* at a dose of 200mg/kg and 400mg/kgto protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

Table no: 1 illustrates the levels of initial and final blood glucose,and change in body weight , in normal rat,and treatment control animals in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with *KSP*at a dose of 200mg/kg and 400mg/kg was increased the body weight non-significantly as compared to normal control animals.

Fasting blood glucose level was significantly increased 210.45 ±6.70 in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range indiabetic rats treated with *KSP*at a dose of 200mg/kg and 400mg/kg.

Table no: 2 illustrates the levels oftotal hemoglobin, glycosylated hemoglobin and plasma insulin in normal ratand treatment control animals in each group.

The levels of total hemoglobin, and plasma insulin levels were decreased significantly where as glycosylated heamoglobin levels were increased significantlyas compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin,returned to near normal range indiabetic rats treated with *KSP* at a dose of 200mg/kg and 400mg/kg. It reveals the dose dependent efficacy of the trail drug.

**HISTOPATHOLOGY:**

Group I:The normal section of pancreas are seen

GroupII:The pancreas section showing the damaging Islets of langerhans cell seen

GroupIII:Normal pancreas Islets of langerhans are seen compare to SPZ damaging pancreas

Group IV:The normal pancreas islets of langerhans are seen compare to standard drug.

Group V:The normal pancreas islets of langerhans are seen compare to low dose of KSP.

## EFFECT OF *KSP* ON BODY WEIGHT OF TRITON-INDUCED ANTI DYSLIPIDEMIC RATS

**Table 18: Effect of *KSP* on body weight of Triton-induced anti dyslipidemic rats**

| Groups | Body Weight (gm.) | | | | |
|---|---|---|---|---|---|
| | Initial | 1st Week | 2nd Week | 3rd Week | 4th Week |
| G1 Normal control | 155±0.87 | 157±1.3 | 159.25 ±1.03** | 161.5 ±2.72** | 163.6±3.17** |
| nti dyslipidemic Control | 157.17±0 .87 | 159±1.04 | 170.17 ±1.7 | 198.5 ±0.66 | 216±1.1 |
| G3 Lovastatin (10mg/kg/day) | 156±1.59 | 159.17±1.0 8 | 164.5 ±1.18** | 163.17 ±0.91** | 167.5±0.8** |
| KSP250mg/kg | 155.63±0 .9 | 158±0.97 | 164.75 ±1.47* | 167.42 ±1.23** | 170.83±2.3** |
| KSP 500mg/kg | 156.5±0. 67 | 159.5±1.89 | 164.33 ±0.80* | 164.67 ±0.76** | 168.83±0.7** |

*Values are as mean ± SEM (n=6). Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001. Comparison made between Group II Vs Group I and Group III, IV, V Vs Group II.*

**Effect of *KSP* on body weight of Triton-induced anti dyslipidemic rats**

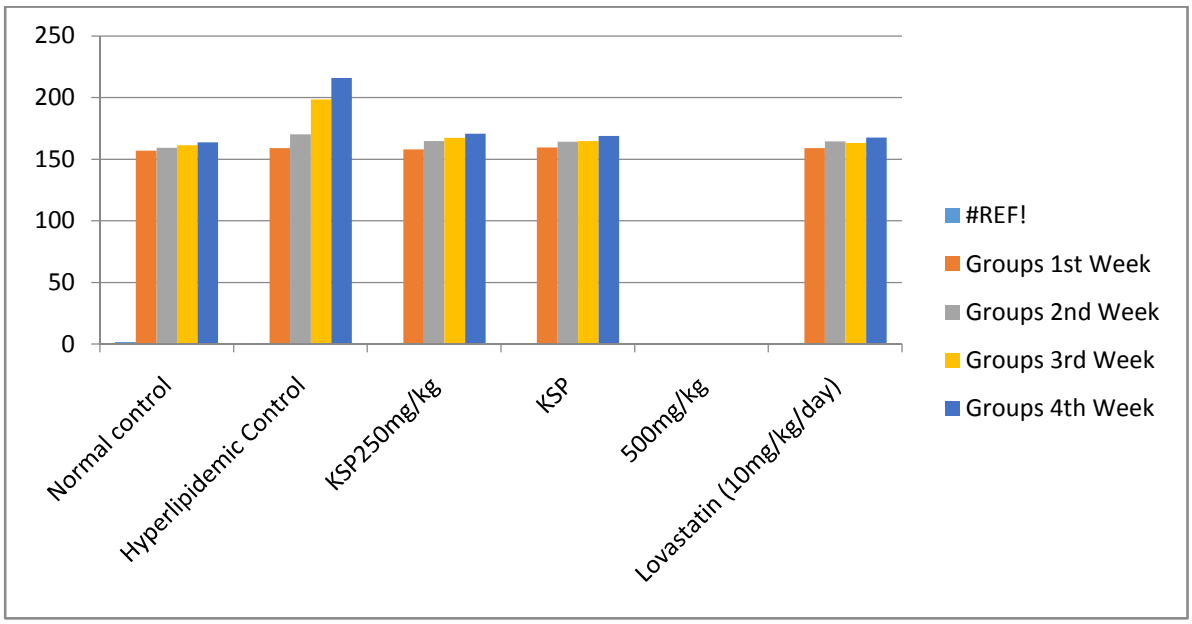



**Fig No: 15 Effect of *KSP* on body weight of Triton-induced anti dyslipidemic rats**

**Table 19: Effect of *KSP on*Blood lipid profile of Triton-induced anti dyslipidemic rats.**

| Group | Treatment | T.C. | T.G. | LDL | HDL | VLDL |
|---|---|---|---|---|---|---|
| I | Normal Control | 72.81±1.1** | 60.17±1.7** | 52.83±1.8** | 35.3±1.3 | 19.3±1.2** |
| II | Triton Control | 174.17±1.35 | 115.17±1.49 | 120.17±1.25 | 28.17±1.08 | 20.83±1.11 |
| III | KSP 250mg/kg | 72.5±1.23** | 69.67±0.99** | 71.17±1.38** | 40.33±0.88* | 14.33±0.80** |
| IV | KSP 500mg/kg | 67.30±1.45** | 56.83±0.90** | 56.33±1.02** | 42.17±1.14** | 11.17±1.25** |
| V | Lovastatin | 54.5±1.12** | 54.3±1.30** | 46.33±1.15** | 44.33±1.36** | 12.5±1.87** |

*Values are as mean ± SEM (n=6). Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001. Comparison made between Group II Vs Group I and Group III, IV, V Vs Group II.*

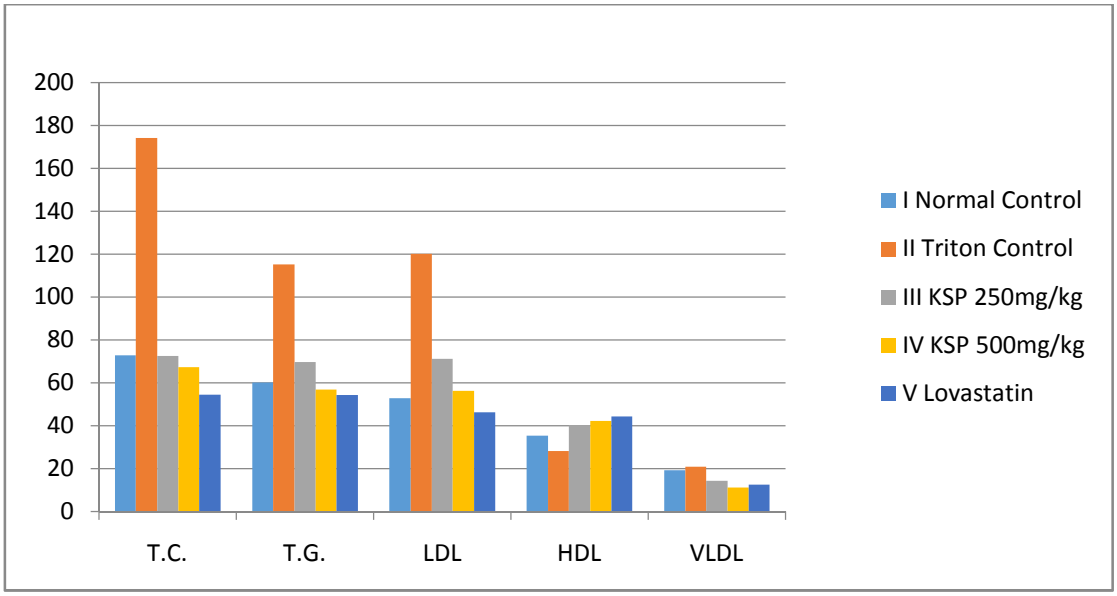



**Fig No: 16 .Effect of *KSP on*liver lipid profile**

**Effect of *KSP on*liver lipid profile of Triton-induced anti dyslipidemic rats.**

| Group | Treatment | T.C. | T.G. | LDL | HDL | VLDL |
|---|---|---|---|---|---|---|
| I | Normal Control | 74.17±0.87** | 70.75±1.93** | 26.17±1.70** | 36.17±0.91 | 13.58±1.24** |
| II | Triton Control | 155.17±1.08 | 178.82±1.46 | 105.25±2.42 | 18.15±1.05 | 35.1±0.93 |
| III | KSP250mg/kg | 82.15±0.92** | 64.15±1.03** | 23.12±1.01** | 35.9±0.86 | 12.22±0.25** |
| IV | KSP 500mg/kg | 75.3±0.85** | 64.12±0.97** | 20.3±1.06** | 44.05±1.4* | 12.88±1.12** |
| V | Lovastatin (10mg/kg/day) | 67.05±0.97** | 57.25±1.15** | 17.93±1.31** | 50.1±0.99* | 12.1±0.91** |

*Values are as mean ± SEM (n=6). Values are statistically significant at *P<0.05, **P<0.01,***P<0.001. Comparison made between Group II Vs Group I and Group III, IV, V Vs Group II.*

**Table 20: Effect of *KSP on*liver lipid profile of Triton-induced anti dyslipidemic rats.**

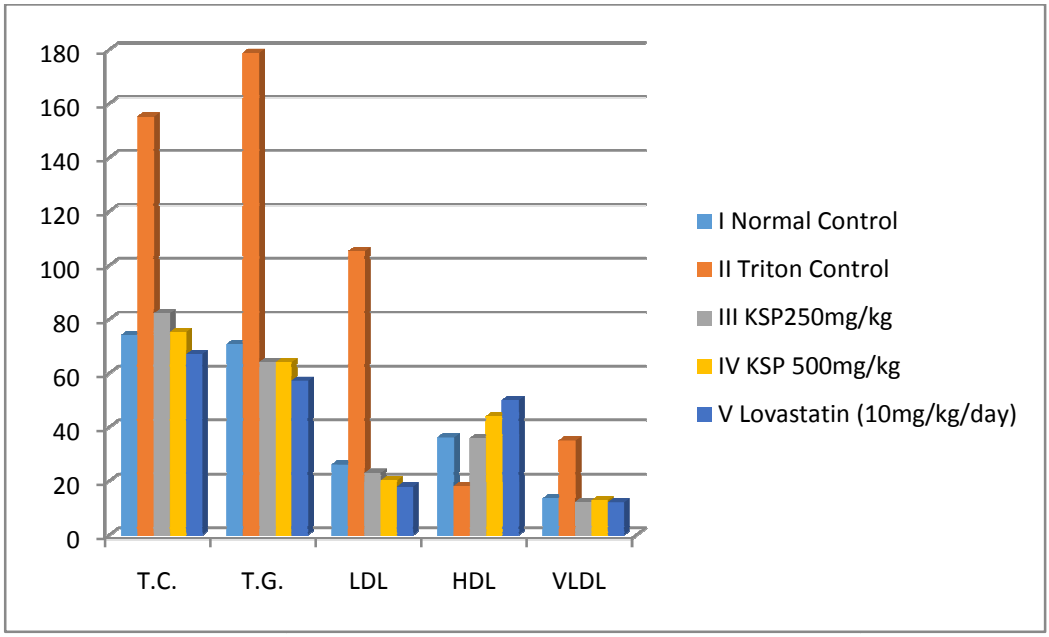



**Fig No: 17: Effect of *KSP* on liver lipid profile of Triton-induced anti dyslipidemic rats.**

## EFFECT OF KSP ON ATHEROGENIC INDEX AND PERCENTAGE PROTECTION OF DIFFERENT GROUPS.

**Table 21: Effect of KSP on atherogenic index and percentage protection of different groups.**

| Groups | Atherogenic Index | % Protection |
|---|---|---|
| **Normal control** | 1.50±0.02 | ---- |
| **Anti dyslipidemic Control** | 5.90±0.10 | ---- |
| **KSP 250mg/kg** | 0.77±0.01 | |
| **KSP 500mg/kg** | 0.72±0.02 | |
| **Lovastatin** | 1.18±0.02 | |

*Values are as mean ± SEM (n=6). Values are statistically significant at *P<0.05, **P<0.01,***P<0.001. Comparison made between Group II Vs Group I and Group III, IV, V Vs Group II.*

## EFFECT OF *KSP ON* SGOT, SGPT TOTAL PROTEIN, UREA AND BLOOD GLUCOSE LEVELS OF TRITON-INDUCED ANTI DYSLIPIDEMICRATS

| Groups | SGOT(U/I) | SGPT(U/I) | Tota Protein (gm/dl) | Urea (mg/dl) | Blood Glucose (mg/dl) |
|---|---|---|---|---|---|
| **Normal control** | 148.17±1.08 | 61.17±0.87 | 5.9±0.86* | 36±0.97 | 85.83±0.79 |
| **Anti dyslipidemic Control** | 190.33±1.12 | 120.5±0.76 | 6.0±0.97 | 45±1.1 | 92.14±0.74 |
| **KSP 250mg/kg** | 176.5±1.67 | 71±2.03 | 5.68±1.08* | 32.02±1.1 | 86.83±1.08 |
| **KSP 500mg/kg** | 150±1.18** | 70.37±0.89** | 5.17±1.02 | 32.6±1.2** | 85.08±1.30** |
| **Lovastatn (10mg/kg/day)** | 155.52±1.74 | 64.05±1.35 | 5.13±0.90 | 28.67±0.6 | 85.17±0.87 |

*Values are as mean ± SEM (n=6). Values are statistically significant at *P<0.05, **P<0.01,***P<0.001. Comparison made between Group II Vs Group I and Group III, IV, V Vs Group II.*

**Table 22: Effect of *KSP on*SGOT, SGPT Total protein, Urea and Blood glucose levels of Triton-induced anti dyslipidemicrats**

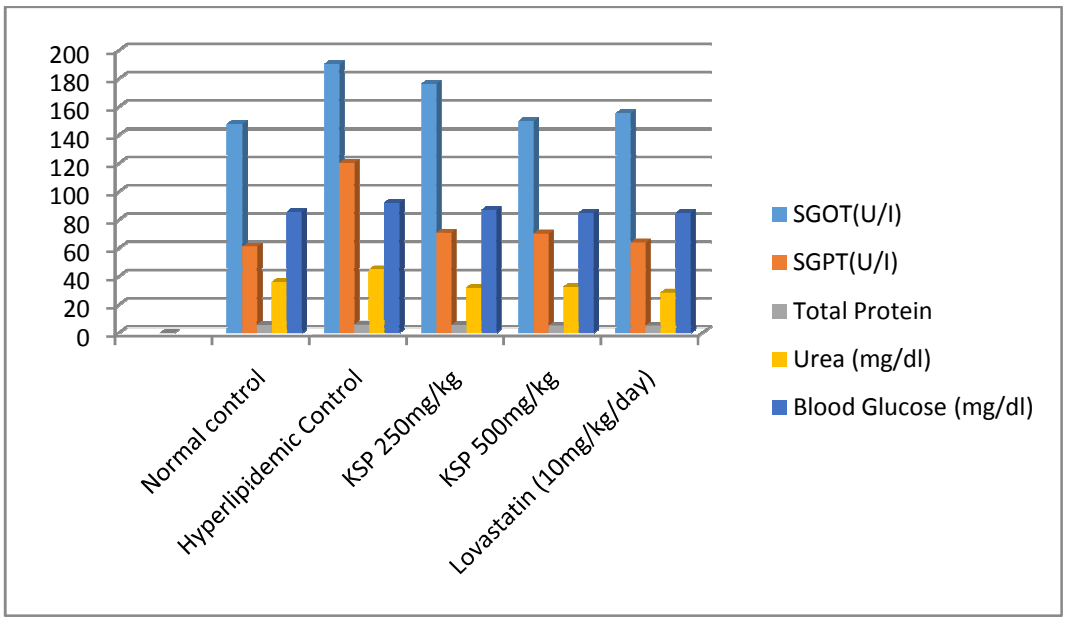
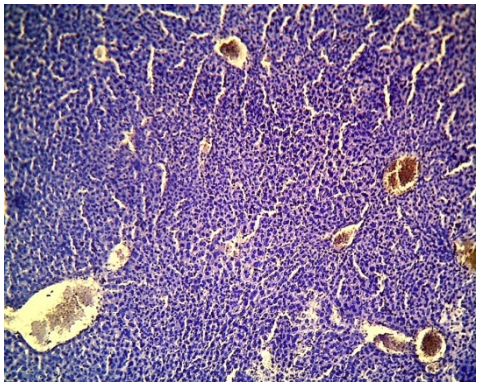


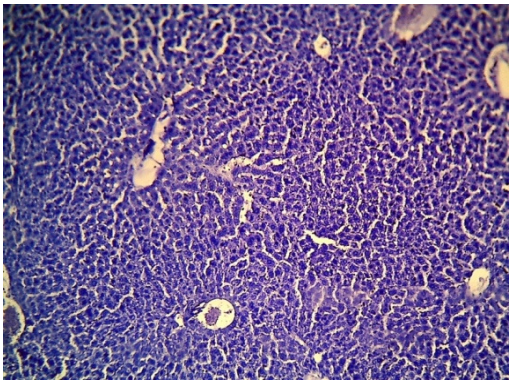

**Fig No 18: Effect of *KSP on*SGOT, SGPT Total protein, Urea and Blood glucose levels of Triton-induced anti dyslipidemicrats**

**Fig No:19.**

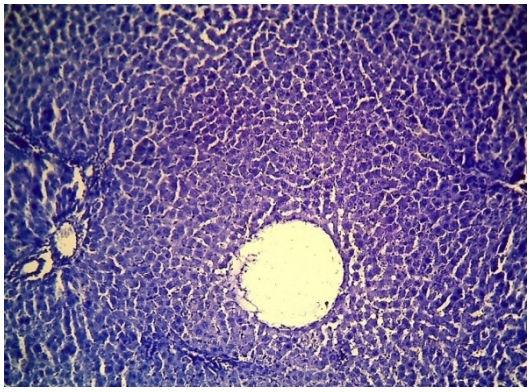
**HYPER LIPIDEMIC – NORMAL**



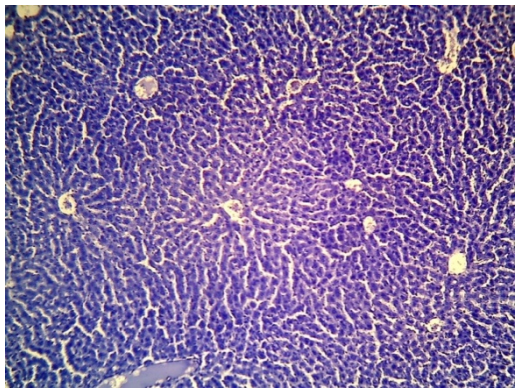
**CHOLESTEROL INDUCEED**



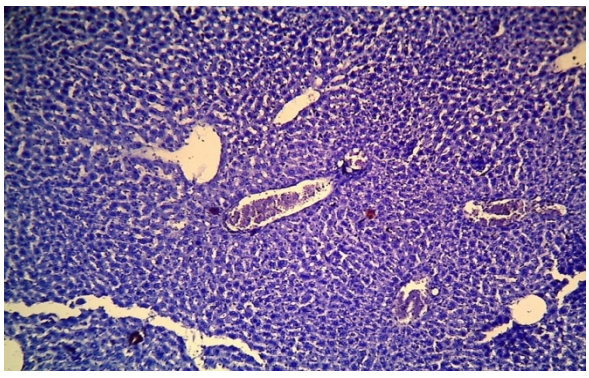
**ANTI DYSLIPIDEMIC – STD/CONTROL**



**ANTI DISLIPIDEMIC –LOW DOSE**



**ANTI DISLIPIDEMIC – HIGH DOSE**



**RESULT :**

The level of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coronary heart disease (CHD). Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

Triton has been widely used to block the clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs. The results showed that *KSP* produced a significant reduction in cholesterol level and also it reversed Triton induced hypolipidemic in rats. Similarly, *KSP* at a dose of 250 and 500mg/kg significantly lowered both plasma triglycerides and cholesterol levels. The reduction of total cholesterol by the *KSP* at the dose level of 250 and 500 mg kg may be associated with a decrease of LDL, which is the ultimate aim of many hypolipidemic agents.

This study suggests that cholesterol-lowering activity of the *KSP* may increase the fecal excretion of bile acids and neutral sterols with the consequent reduction of hepatic cholesterol because of its use in the biosynthesis of these bile acids. These fractions also slow down the rate of diffusion through the intestinal mucosa thereby reducing the absorption of cholesterol and triglycerides. Anti oxidant constituents of *Kadukkai*, *Nellimuli* and *thandrikai* also prevent the endogenous oxidation of cholesterol result in decrease in the concentration of low density lipoprotein and again confirms the hypolipidemic drug.

Table no:19 & 20 shows the KSP has significant anti-dyslipedemic activity and the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL),Low density lipoprotein(LDL) and phospholipids of normal and experimental animals in each group.

The main ‘anti-atherogenic’ lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver and thereby it acts as a protective factor against coronary heart disease (CHD).

**HISTOPATHOLOGY:**

| | | |
|---|---|---|
| Group I | : | Normal section of hepatic cells are seen. |
| Group II | : | Showing degeneration of hepatocytes in some were portal zone.This appear more eosinophilic. |
| Group III | : | Showing normal features of hepatic tissue compared to cholesterol induced group. |
| Group IV | : | Showing normal feature of hepatic tissue compared to standard group. |
| Group V | : | Normal hepatic cells are seen compared to low dose of KSP. |

## IN VITRO ANTIOXIDANT ACTIVITY OF *KSP* ON DPPH RADICAL SCAVENGING METHOD

| Sample concentration (µg/ml) | Ascorbic acid | | *KSP* | |
|---|---|---|---|---|
| | Drug | Standard | Drug | Standard |
| Control | 0.5441 | 0.354 | - | - |
| 1.25 | 0.4921 | 0.262 | 9.5% | 25% |
| 2.50 | 0.3825 | 0.182 | 29% | 48% |
| 5 | 0.2782 | 0.104 | 48% | 70% |
| 10 | 0.1820 | 0.082 | 66% | 76% |
| 20 | 0.1025 | 0.076 | 81% | 78% |

*µg/ml: microgram per millilitre. Drug: KSP (1.25-20µg/ml). Standard: Ascorbic acid (10mg/ml DMSO)*

**Table No.23. Anti-oxidant activity of KSP**

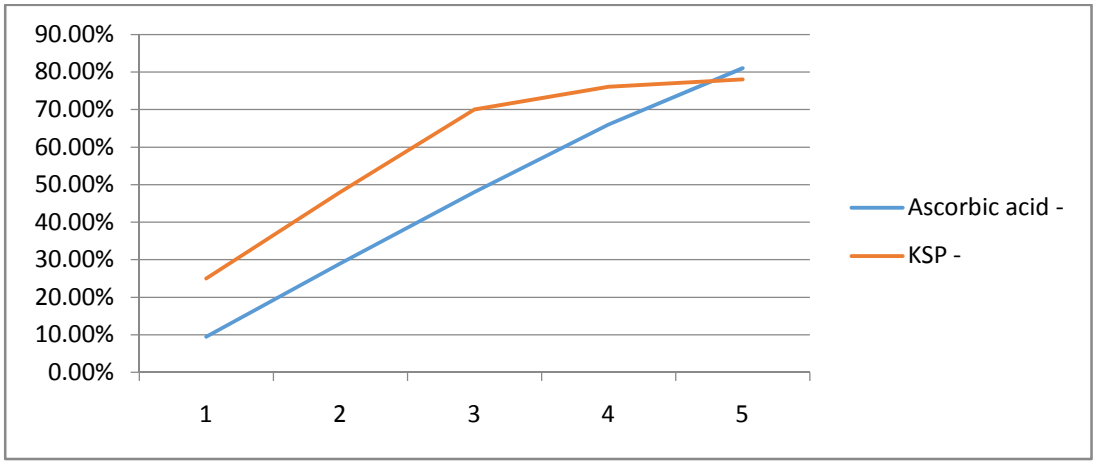



**Fig No:20: GRAPICAL REPRESENATION**

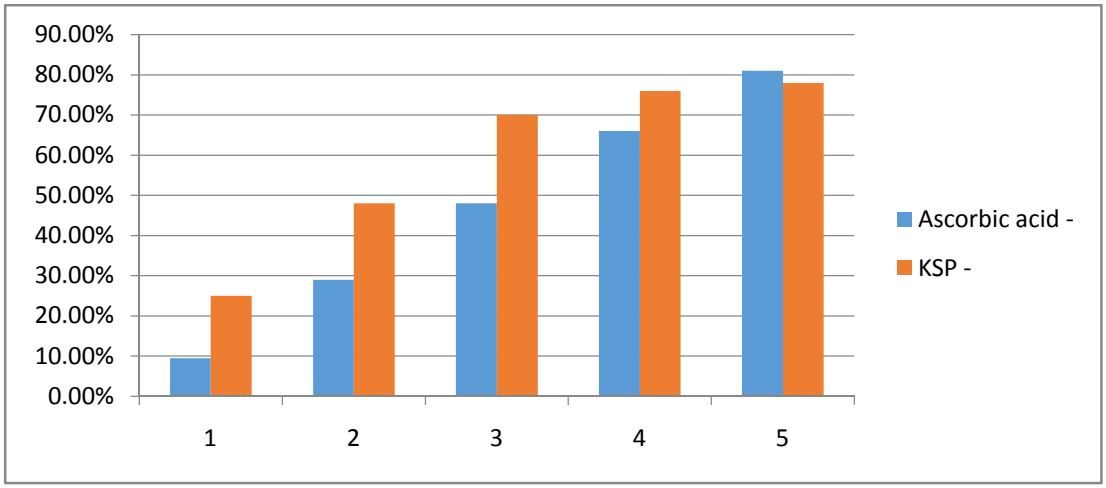



**FIG No.21. Anti-oxidant activity of KSP**

**INTERPRETATION:**

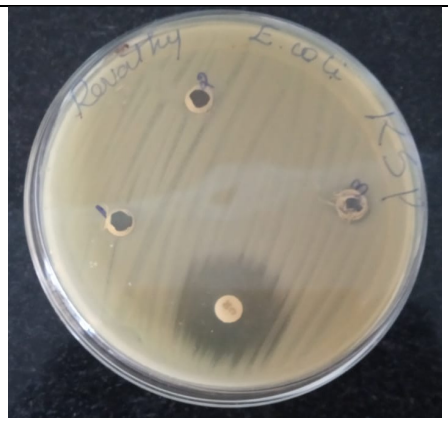
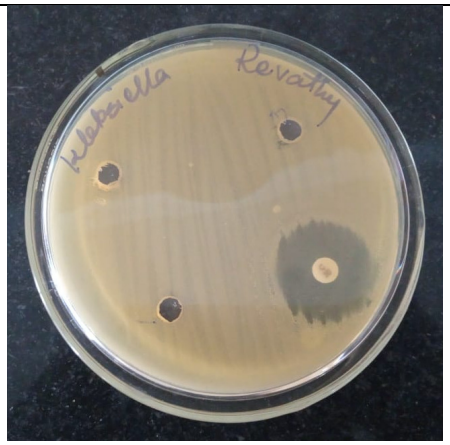
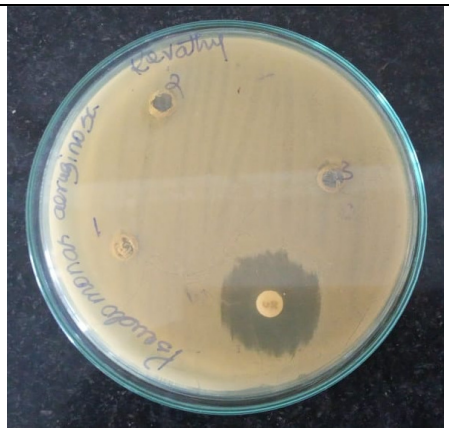
Antioxidants slow down the oxidative damage of our body. Antioxidants act as a freeradical scavengers, Preventing and repairing damages. Health problems such as Heart diseases, cancer and degenerative disorders are all exacerbated by oxidative damage. The Antioxidant property of the drug *KSP* was tested by DPPH assay. The results showed that there was a concentration dependent Antioxidant property of crude extract of *KSP*. At the concentration increased from 1.25 to 10 µg/ml, percentage of inhibition increased from 48 % to 78 %. At a concentration of 100 µg/mlthere was an increased percentage of inhibition (78 %) in scavenging the free radicals (DPPH). The IC50 value was obtained at 25.70 µg/ml. It showed that *KSP* is having significant anti oxidant property. Hypoglycemia promotes auto – oxidation of glucose to form free radicals, the generation of free radicals beyond the scaving abilities of endogenous antioxidant defenses results in macro and mcro vascular dysfunction.

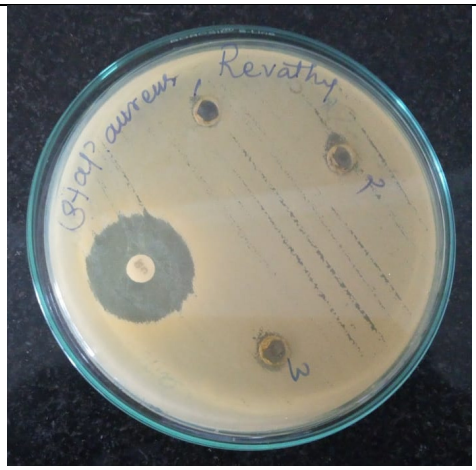
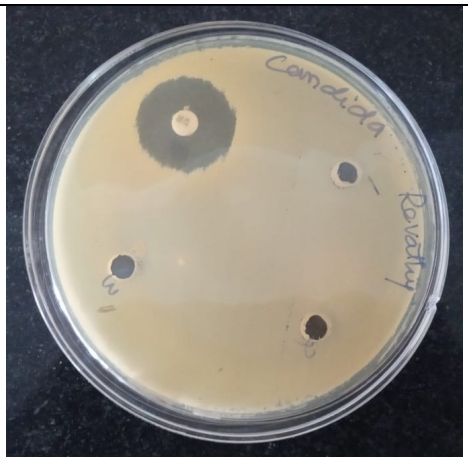
# MICROBIOLOGICAL ANALYSIS

**Table 24. Antimicrobial Activities of Various Samples by Agar Well Diffusion Method**

| S.No. | Bacterial Pathogens | Zone of Inhibition (mm) | | | | Result |
|---|---|---|---|---|---|---|
| | | Positive C-30ontrol | Sample Load (µl) | | | |
| | | | 10 µl | 20 µl | 30 µl | |
| 1. | *Escherichia coli* | Chloramphenicol-30mcg(24 mm) | R | R | R | Resistant |
| 2. | *Klebsiella sps.* | Chloramphenicol-30mcg (24 mm) | R | R | R | Resistant |
| 3. | *Pseudomonas aeruginosa* | Chloramphenicol-30mcg (23 mm) | R | R | R | Resistant |
| 4. | *Staphylococcu aureus* | Chloramphenicol-30mcg (25 mm) | R | R | R | Resistant |
| 5. | *Candida sp* | Ketoconazole-30mcg (24mm) | R | R | R | Resistant |

**Fig No: 22 Microbiological Analysis of *KSP***

| S.No. | Bacterial Pathogens | Plates |
|---|---|---|
| 1. | *Escherichia coli* |  |
| 2. | *Klebsiellasps.* |  |
| 3. | *Pseudomonas aeruginosa* |  |

| | | |
|---|---|---|
| 4. | *Staphylococcu aureus* |  |
| 5. | *Candida sp* |  |

**INTERPRETATION:**

Both Gram positive and Gram negative bacteria *E.coli, Klebsiella pneumoniae* and *Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi* were found to be resistance when compared to the standard drug Gentamycin (Broad spectrum) . Hence the trail drug *KSP* has not anti microbial activity.

# TOXICITY RESULTS

## EVALUATION OF ACUTE TOXICITY STUDY OF *KSP*

**Table no –25 Physical and behavioral examinations.**

| Group no. | Dose(mg/kg) | Observation sign | No. of animal affected. |
|---|---|---|---|
| Group-I | 5mg/kg | Normal | 0 of 3 |
| Group- II | 50mg/kg | Normal | 0 of 3 |
| Group-III | 300mg/kg | Normal | 0 of 3 |
| Group-IV | 1000mg/kg | Normal | 0 of 3 |
| Group-V | 2000mg/kg | Normal | 0 of 3 |

**Table no-26 Home cage activity**

| Functional and Behavioural observation | Observation | 5mg/kg Group (G-I) | 50 mg/kg (G-II) | 300 mg/kg (G-III) | 1000 mg/kg (G-IV) | 2000 mg/kg (G-V) |
|---|---|---|---|---|---|---|
| | | Female n=3 | Female n=3 | Female n=3 | Female n=3 | Female n=3 |
| Body position | Normal | 3 | 3 | 3 | 3 | 3 |
| Respiration | Normal | 3 | 3 | 3 | 3 | 3 |
| Clonic involuntary Movement | Normal | 3 | 3 | 3 | 3 | 3 |
| Tonic involuntary Movement | Normal | 3 | 3 | 3 | 3 | 3 |
| Palpebral closure | Normal | 3 | 3 | 3 | 3 | 3 |
| Approach response | Normal | 3 | 3 | 3 | 3 | 3 |
| Touch response | Normal | 3 | 3 | 3 | 3 | 3 |
| Pinna reflex | Normal | 3 | 3 | 3 | 3 | 3 |
| Tail pinch response | Normal | 3 | 3 | 3 | 3 | 3 |

**Table no-27 Hand held observation**

| Functional and Behavioral observation | Observation | Control | 5 mg/ kg (G-I) | 50 mg/kg (G-II) | 300 mg/kg (G-III) | 1000 mg/kg (G-IV) | 2000 mg/kg (G-V) |
|---|---|---|---|---|---|---|---|
| | | Female n=3 | Female n=3 | Female n=3 | Female n=3 | Female n=3 | Female n=3 |
| Reactivity | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Handling | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Palpebral closure | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Lacrimation | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Salivation | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Piloerection | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Pupillary reflex | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Abdominal tone | Normal | 3 | 3 | 3 | 3 | 3 | 3 |
| Limb tone | Normal | 3 | 3 | 3 | 3 | 3 | 3 |

**Table no-28 Mortality**

| Group no | Dose no(mg/kg) | Mortality |
|---|---|---|
| Group-I | 5(mg/kg) | 0 of 3 |
| Group-II | 50(mg/kg) | 0 of 3 |
| Group-III | 300(mg/kg) | 0 of 3 |
| Group-IV | 1000(mg/kg) | 0 of 3 |
| Group-V | 2000(mg/kg) | 0 of 3 |

**RESULT:**

From acute toxicity study it was observed that the administration of *KSP* at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of *KSP*is 2000 mg/kg.

## DISCUSSION

***KSP*** was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***KSP*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

Body weight at weekly interval was measured to find out the effect of ***KSP*** on the growth rate. Body weight change in drug treated animals was found normal.

# SUB-ACUTE TOXICITY

**Table :29 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *KSP* ON BODY WEIGHT IN GRAM**

| BODY WEIGHT | CONTROL GROUP | LOW DOSE GROUP | MIDDLE DOSE GROUP | HIGH DOSE GROUP |
|---|---|---|---|---|
| 1st day | 121.3±1.03 | 126±2.544 | 125.4±3.232 | 127.4±3.24 |
| 7th day | 133.4±2.04 | 132.4±2.344 | 132±3.114 | 138±3.21 |
| 14th day | 135.2±2.104 | 103.4±2.22 | 103.5±3.112 | 104.5±3.112 |
| 21st day | 104.4±3.220 | 111.3±2.601 | 105±2.231 | 106±2.23 |
| 28th day | 114.4±2.141 | 113.4±2.302 | 144±3.1405 | 147±3.140 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

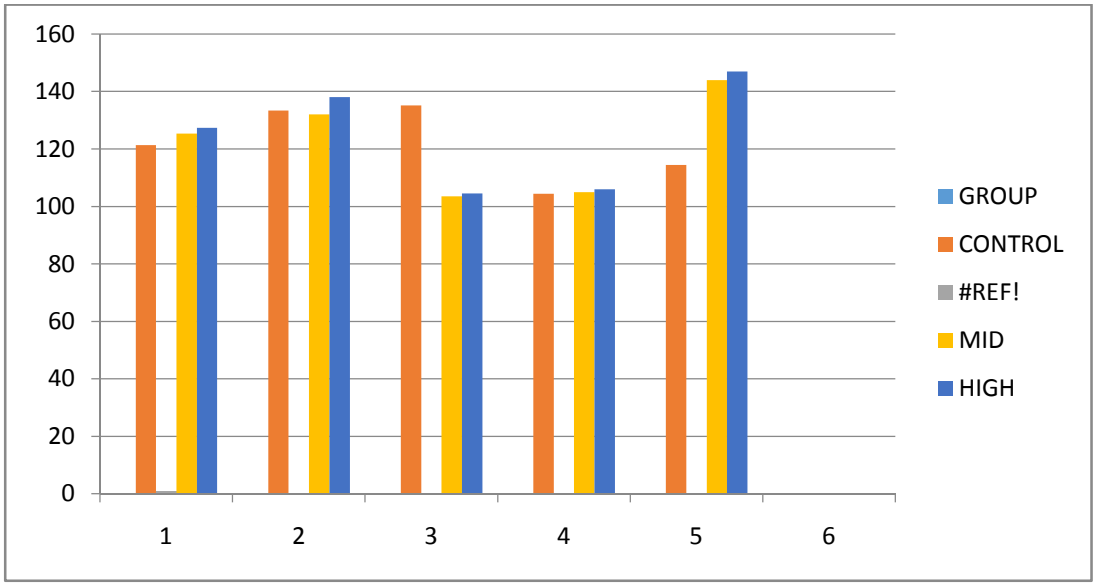



**FIG NO 23: EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *KSP* ON BODY WEIGHT IN GRAM**

## EFFECT OF SUBACUTE DOSE (28 DAYS)OF *KSP*

**Table : 30 *KSP* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM**

| ORGANS | | CONTROL | LOW | MID | HIGH |
|---|---|---|---|---|---|
| HEART | | 0.53±0.03 | 0.34±0.05 | 1.11±0.21 | 0.51±0.03 |
| LIVER | | 3.41± 0.33 | 5.43±0.33 | 3.30±0.02 | 3.33± 0.33 |
| LUNGS | | 2.31±0.10 | 0.41±0.15 | 0.60±0.25 | 2.43±0.20 |
| KIDNEY | L | 0.53±0.03 | 1.62±0.04 | 0.53±0.03 | 0.51±0.03 |
| | R | 0.51±0.025 | 2.14±0.02 | 0.51±0.025 | 0.52±0.025 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

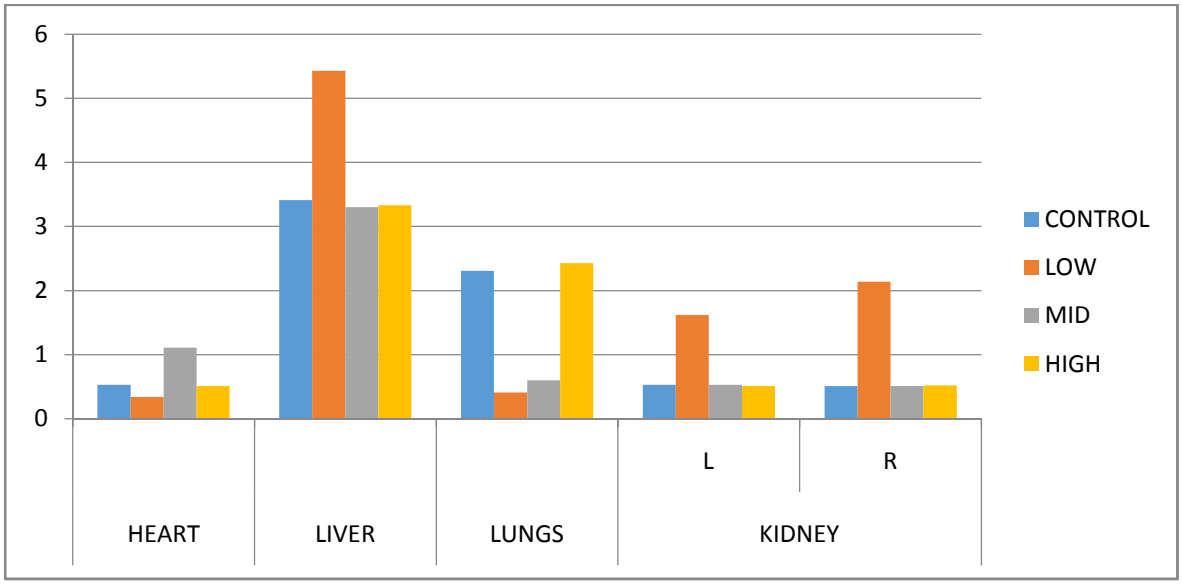



**Fig No: 24 *KSP* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM**

## EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KSP* ON HAEMATOLOGICAL PARAMETERS

**Table no 31: EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KSP* ON HAEMATOLOGICAL PARAMETERS**

| Drug treatment | RBC million cells/cmm | WBC cells/cmm | Haemoglobingm % | Differential count % | | | |
|---|---|---|---|---|---|---|---|
| | | | | Neutophils | Eosinophils | Monocyte | Limpocyte |
| Control | 5.21±0.50 | 4352.41±24.32 | 15.41±0.55 | 41.27±2.20 | 2.53±0.21 | 4.45±0.25 | 33.14±4.32 |
| LOW | 5.47±0.30 | 4434.04±24.22 | 15.20±0.44 | 35.55±2.41 | 3.20±0.24 | 5.22±0.40 | 33.32±4.51 |
| MID | 5.43±0.31 | 4404.26±42.36 | 15.11±1.03 | 40.42±2.32 | 2.44±0.12 | 4.32±0.50 | 33.13±4.33 |
| HIGH | 5.25±0.31 | 588.26±33.36 | 17.12±2.03 | 38.32±3.23 | 2.50±0.22 | 4.35±0.50 | 34.14±4.33 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

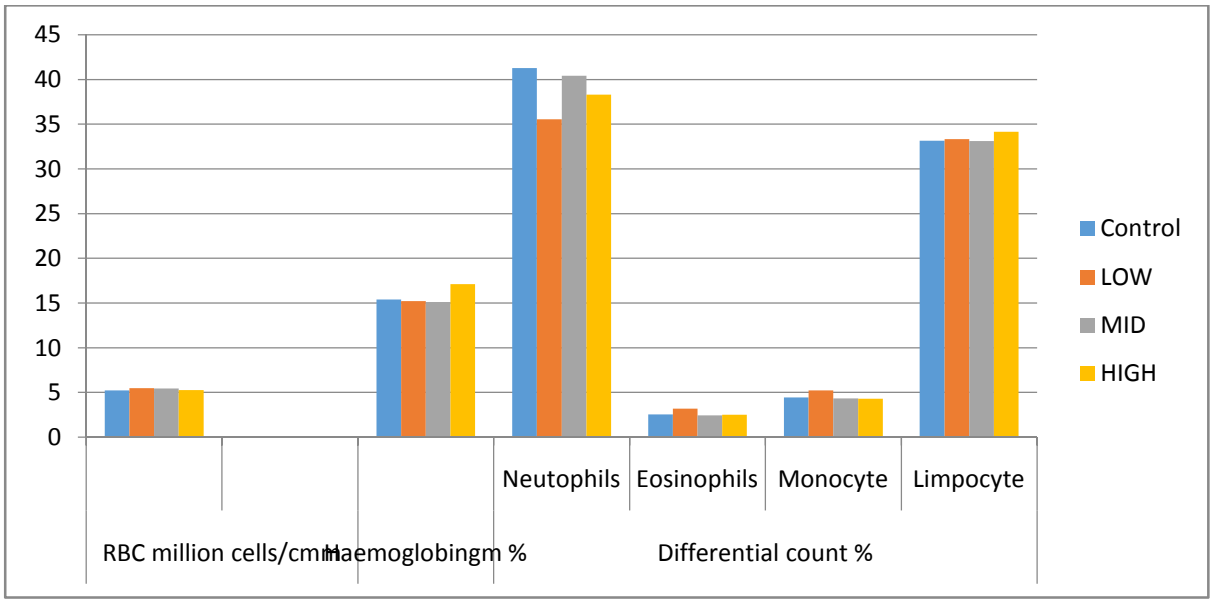



**Fig No: 25: EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KSP* ON HAEMATOLOGICAL PARAMETERS**

**Table :33 EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF *KSP* ON BIOCHEMICAL PARAMETER**

| Drug Treatment | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|---|---|
| Control | 43.24±4.03 | 152.25±5.32 | 253.13±12.33 | 36.36±4.00 | 0.64±0.04 |
| LOW | 43.14±4.23 | 151.24±5.02 | 261.12±13.43 | 41.54±3.43 | 0.60±0.05 |
| MID | 41.23±5.45 | 154.32±3.22 | 255.46±5.15 | 40.13±3.23 | 0.46±0.05 |
| HIGH | 43.22±5.45 | 150.32±3.22 | 244.46±5.15 | 41.13±3.23 | 0.56±0.05 |

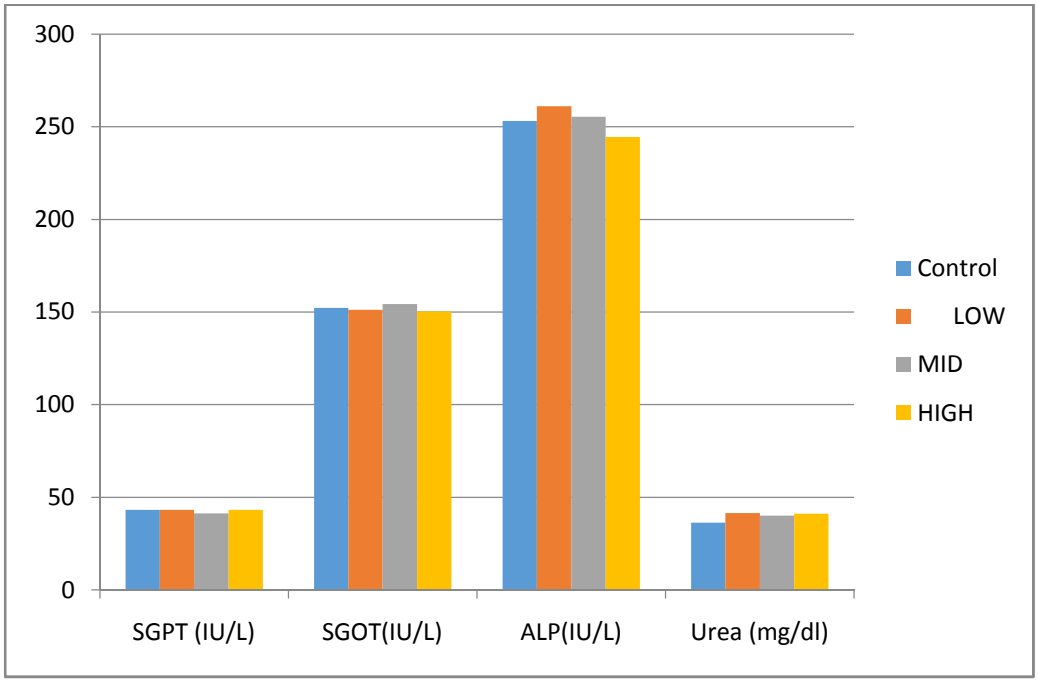



**Fig No: 27 EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF *KSP* ON BIOCHEMICAL PARAMETER**

**Table:34 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KSP***

**BIOCHEMICAL PARAMETERS**

| GROUP | CONTROL | *KSP* (200mg/kg) | *KSP* (400mg/kg) | *KSP* (600mg/kg) |
|---|---|---|---|---|
| TOTAL BILIRUBIN (mg/dl) | 1.308±0.2457 | 1.458±0.2827 | 0.8198±0.3376 | 0.104 ±0.199 |
| CREATININE (mg/dl) | 0.6133±0.03849 | 0.63±0.08827 | 0.47720.0446 | 0.5872 ±0.02528 |
| URIC ACID (mg/dl) | 1.752±0.2766 | 1.79±0.160 | 2.06±0.6617 | 1.988 ±0.2929 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}$p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

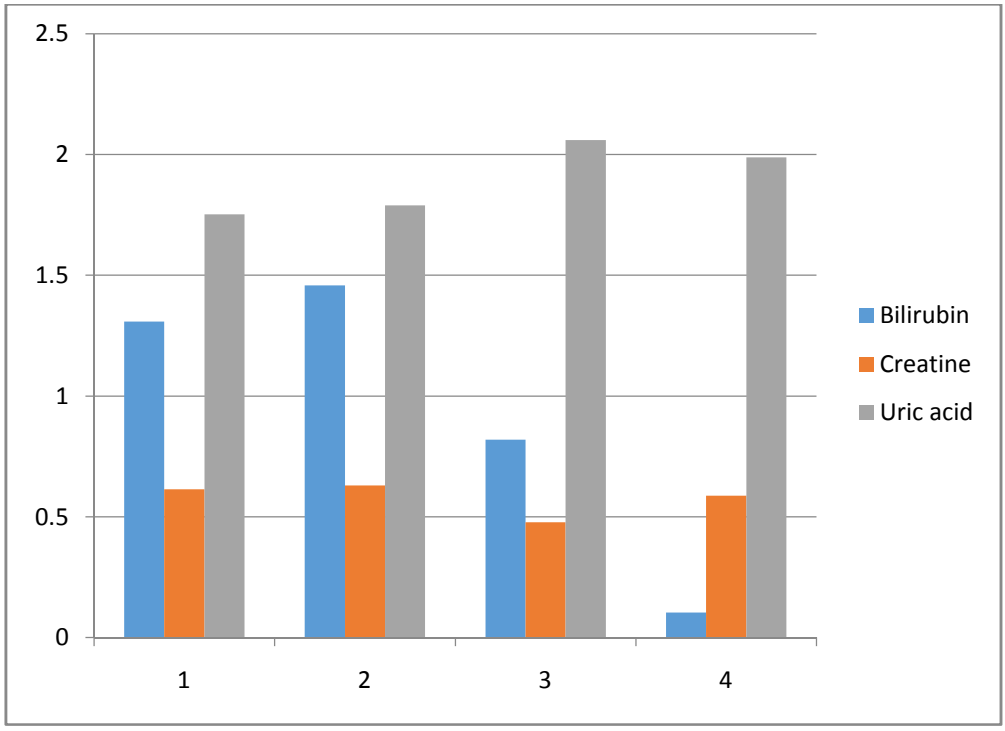



**Fig No:28 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KSP* *ON***

**BIOCHEMICAL PARAMETERS**

**Table No:34 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OFON FOOD INTAKE IN GRAM**

| GROUP | CONTROL | *KSP* (200mg/kg) | *KSP* (400mg/kg) | *KSP* (600mg/kg) |
|---|---|---|---|---|
| 1st DAY | 19.33±13.6110 | 20.1672±15.3 | 13.10±22.71 | 18.5±8.62 |
| 7th DAY | 16.5±12. | 11.863±13.67 | 17.73±10.853 | 12.17±15.41 |
| 14th DAY | 19.83±8.72 | 11.83±15.28 | 11±14.96 | 20.72±9.981 |
| 21st DAY | 12.87±13.4 | 16±8.466 | 16.88±10.43 | 20.17±9.02 |
| 28th DAY | 13.10±11.38 | 19.38±11.50 | 11±8.90 | 11±7.57 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group

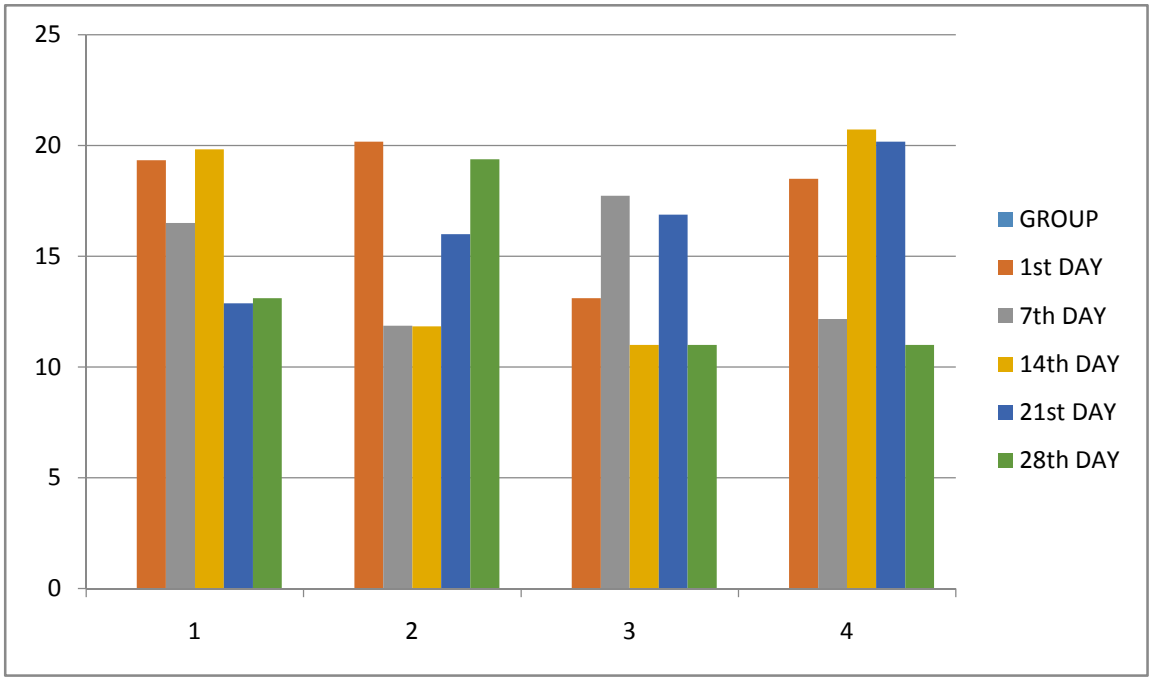



**Fig No: 28 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OFON FOOD INTAKE IN GRAM**

**Table No: 35 Effect of Sub- Acute Dose (28 Days) Of *KSP*On Water Intake in ml**

| GROUP | CONTROL | *KSP* (200mg/kg) | *KSP* (400mg/kg) | *KSP* (600mg/kg) |
|---|---|---|---|---|
| 1st DAY | 98.3338±13.5110 | 89.1672±14.3426 | 102.10±21.7199 | 67.5±7.6203 |
| 7th DAY | 85.5±11.7938 | 100.863±12.6770 | 76.6673±9.85363 | 81.6717±14.4150 |
| 14th DAY | 58.3383±8.72817 | 90.8363±14.2812 | 80±13.9692 | 89.1672±8.88981 |
| 21st DAY | 91.6687±12.4949 | 85±8.46662 | 65.8338±9.43550 | 89.1717±8.79602 |
| 28th DAY | 82.10±11.3840 | 88.3348±11.5004 | 80±8.90061 | 70±7.57773 |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group

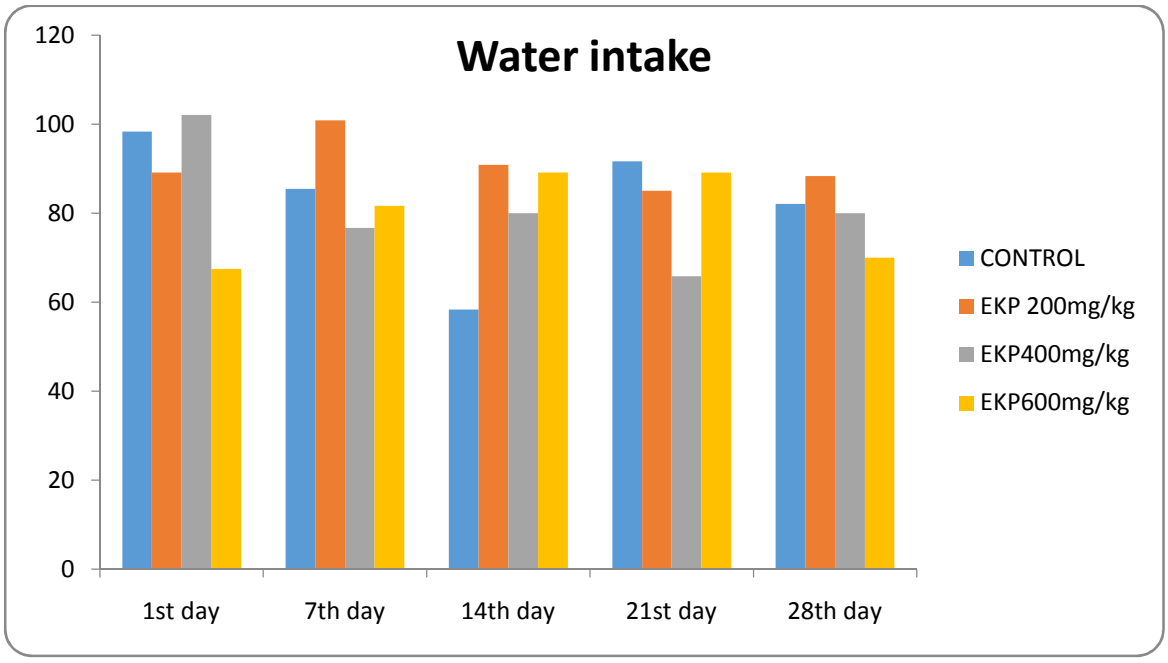



**Figure:29. Effect of Sub- Acute Dose (28 Days) Of *KSP*On Water Intake in ml**

**Table No: 36 EFFECT OF SUB ACUTE DOSES (28 DAY) OF *KSP* ONELECTROLYTES: -**

| GROUP | CONTROL | *KSP* (200mg/kg) | *KSP* (400mg/kg) | *KSP* (600mg/kg) |
|---|---|---|---|---|
| Sodium (mg/dl) | 4.10±0.6855 | 4.30±0.6792 | 11±0.7571 | 11.80±0.70 |
| Calcium(mg/dl) | 1.580±0.137889 | 3.20±0.175783*** | 4.7±0.165299*** | 6.180±0.19611*** |
| Phosphorus (U/L) | 0.278±0.023017 | 0.3010±0.019915$^{ns}$ | 0.35630±0.035491$^{ns}$ | 5.037±0.32502* |

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001,

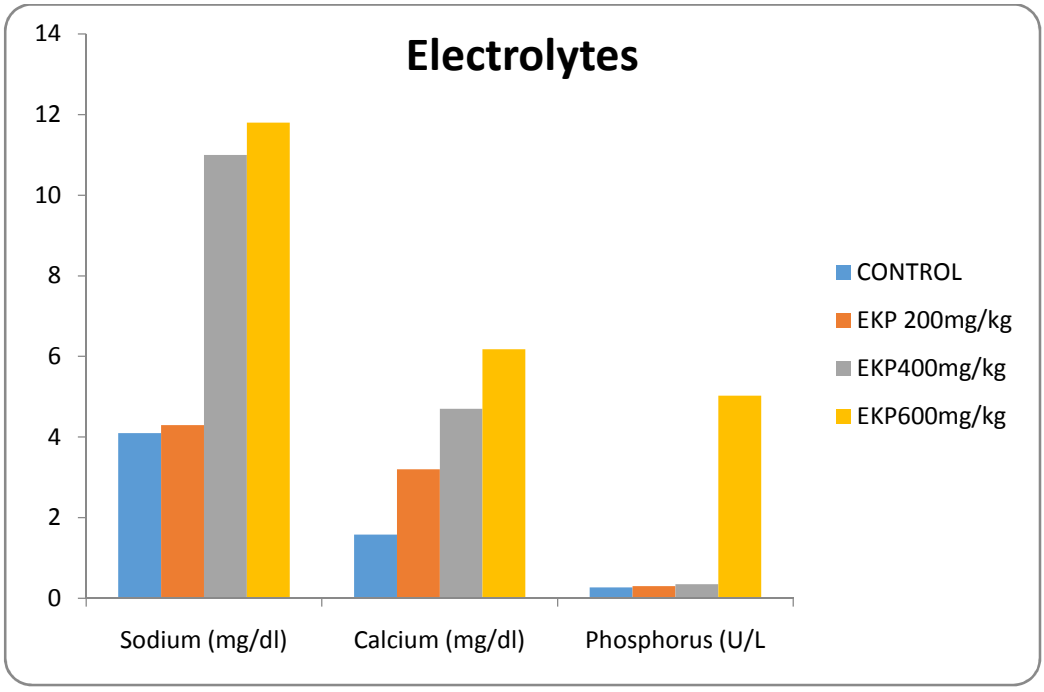



**Figure no.30**

# RESULTS

## CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

**Mortality:**

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

**Body weight:**

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

**Food consumption:**

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

**Organ Weight:**

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.22 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

**Hematological investigations:**

The results of hematological investigations conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits.

**Biochemical Investigations:**

Results of Biochemical investigations conducted on the day 29th and recorded in Table no 24, 25 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

**INTERPRETATION**:

1) All the animals from control and all the treated dose groups up to 2000mg/kg survived throughout the dosing period of 28 days.
2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
5) Haematological analysis conducted at the end of the dosing period on day 29th, revealed no abnormalities attributable to the treatment.
6) Biochemical analysis conducted at the end of the dosing period on day $29^{th}$, no abnormalities attributable to the treatment.
7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

# 6. SUMMARY

The trial drug "*Komoothira silasathu parpam*" was selected from the text "*Thanjai Vaithiya Raja Sinthamani"* for the validation of safety, efficacy and its potency in hypoglycemic, Anti dyslipidemic & anti oxidant activities

The raw drugs *Komoothira silasathu, Kadukkai, Nelli Mulli, Thandrikai* were purchased from Ramasamy Chetty Traders, Paris, Chennai. These raw drugs were identified and authenticated by experts of PG Gunapadam department,Govt.siddha medical college, Palayamkottai.

The trial drug was prepared as per the standard operating procedure.

Various literary collections of Siddha and modern sciences about the ingredients of the trial drug supported the fact that *Komoothira silasathu parpam* has good effect in *madhumegam*.

As in the quote of "பகர்பித்த விந்தையலாது மேகம் வராது" mega disease is caused by derangement of *pitha* humour. Literary evidences show that *madhumegam* comes under the *pitha* variety of *meganoi* 20.

KSP is in astringent taste. Astringent normalized the *pitha* humour which is the basic cause of *madhumegam.* It is described as

"குருதி சுத்தியாக்கும்
கொடிய பித்தம் போக்கும்"

The results Physico-chemical analysis confirm that the *Komoothira silasathu parpam* is light white in colour and low moisture content and did not contain free metals or microbes. This ensuring the safety of the trial drug.

Bio Chemical analysis revealed the presence of calcium, sulphate, chloride, ferrous iron,amino acid

ICP-OES reveals high concentration of physiologically important minerals like, calcium phosphorus, potassium, magnesium, sodium, sulphur study and also it reveals below detection limit(BDL) of heavy metals As (arsenic), Hg (Mercury), Cd(cadmium), Pb(lead) and Cu(copper). This result also indicates that the formulation KSP is extremely safe as it contains heavy metals within WHO specified limits.

Calcium release insulin hormone from the islets of Langerhans.

Potassium is required for the transmission of nerve impulse which control numbness.

Phosphorus is important for energy transfer in cells as part of ATP and is found in many other biological important molecules. Thus it relieves fatigue which is one of the symptom of *madhumegam.*

Eating a diet with more magnesium is linked with a reduced risk of diabetes in adults.

FTIR confirms that *Komoothira silasathu parpam* constitutes Alkane, aldehyde, Amines, Alcohols.

SEM analysis of the *Komoothira silasathu parpam* shows the particle size varies between 5µm to 10µm. The surfaces of the sample grains is uniformly arranged in agglomerates. Smaller sized particles enhance the absorption and the bioavailability of the drug resulting efficacy of the drug will be increased.

Hypoglycemic activity results showed, fasting blood glucose level is reduced from 190.66 mg/dl to 178.25mg/dl in the dose 100mg of KSP and it was further decreased to 164.25mg/dl in the dose of 200mg of KSP and this reveals dose dependent decrease of blood glucose. Despite the severity of the disease, administration of *Komoothira silasathu parpam* succeeded in significantly reducing blood glucose level in a good manner.

In DM , dyslipidemic is common. In rats treated with both doses of Siddha formulation KSP and Triton there was significant decrease in the content cholesterol TGs, LDL , VLDL and increases HDL when compared with cholesterol controlled rats. KSP has significant Anti dyslipidemic effect.

The Antioxidant property of the drug *Komoothira silasathu parpam* was tested by DPPH assay. The results showed that there was a concentration dependent Antioxidant property of *Komoothira silasathu parpam.* At the concentration increased from 10 to 100 µg/ml, percentage of inhibition increased from 50 % to 78 %. At a concentration of 100 µg/mlthere was an increased percentage of inhibition (78 %) in scavenging the free radicals (DPPH). It showed that *komoothira silasathu parpam* is having significant anti oxidant property.

The drug *Komoothira silasathu parpam* has no antimicrobial activity to Salmonella typhi, Staphylococcus aureus, Escherchia coli, pseudomonas.

From the acute toxicity study ( OECD guidelines 423), it was observed that the administration of *Komoothira silasathu parpam* at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. The test drug *Komoothira silasathu parpam* is a safety drug.

No toxic effect was observed up to 600 mg/kg of *Komoothira silasathu parpam* treated over a period of 28 days (OECD 407). So, *Komoothira silasathu parpam* can be prescribed for therapeutic use in human being in the recommended dosage for prolonged period.

Based on the results presented in this study, it can be concluded that *komoothira silasathu parpam* exerts significant Hypoglycemic, Anti dyslipidemic and Antioxidant activities. Further studies are needed to isolate the active constituents and also to evaluate the exact mechanism of action.

# 7. CONCLUSION

It is concluded that the test drug *Komoothira silasathu parpam* has significant hypoglycemic, anti dyslipidemic and anti-oxidant activities. The anti-oxidant property of the test drug shows it is a preventive medicine and inhibits blood vessel damage. And the test drug KSP is significantly validated by modern techniques and siddha standard method for madhumegam. The toxicological study of this test drug KSP establish the safety of the drug for long time administration. So the test drug of KSP hopefully use for human trials.

# 8. FUTURE SCOPE

The trial drug ***Komoothira silasathu parpam*** has its own potency in treating diabetes mellitus in animal model which has been established in this study. However, the mechanism of action by which ***Komoothira silasathu parpam*** produced its effect on the diabetes mellitus is needed further study to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of diabetes mellitus.

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